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PALLADIN'S

PLANT PHYSIOLOGY

EDITED BY

BURTON E. LIVINGSTON

Prof. V. I. Palladin
1911

PLANT PHYSIOLOGY

BY

VLADIMIR I. PALLADIN

PROFESSOR IN THE UNIVERSITY OF PETROGRAD

AUTHORIZED ENGLISH EDITION

Based on the German Translation of the Sixth Russian Edition and on the Seventh Russian Edition (1914)

EDITED BY

BURTON EDWARD LIVINGSTON, Ph. D.

PROFESSOR OF PLANT PHYSIOLOGY AND DIRECTOR OF THE LABORATORY OF PLANT PHYSIOLOGY OF THE JOHNS HOPKINS UNIVERSITY

Second American Edition

With a Biographic Note and Chapter Summaries by the Editor

173 ILLUSTRATIONS

PHILADELPHIA

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A NOTE OF APPRECIATION[1]

Doctor V. I. Palladin, Academician of the Russian Academy of Sciences and author of this book, died in Petrograd on February 3, 1922, after a prolonged illness that culminated in aortic aneurism. His life work was a fine contribution to physiological science in general, and especially to plant physiology. His text-book on plant physiology was published in Russian, German, French, and English, and the marked excellencies of the book have made his name well known wherever this science is studied. But his greatest contribution lies in his research publications.

Palladin was born July 11, 1859, in Moscow and received his education in the First Gymnasium of Moscow and in the University of Moscow. He studied botany under Timiriazev and Gorozhankin, and published his first research contribution, "On the structure and capacity for swelling of cell walls and starch grains," in 1883 (Zapiski Moskovskogo Univ.). His dissertation for the master's degree, conferred at the University of Moscow in 1887, is on "The significance of oxygen for plants" (Bull. Soc. Nat. Moscow, 1886), and that for the doctor's degree, conferred at the same university in 1888, is on "The influence of oxygen on the decomposition of proteinaceous substances in plants" (Dissert. Moscow Univ., 1889).

The first teaching position held by the great Russian physiologist was in the Institute of Rural Economics and Forestry at Novaya Alexandria, whither he went in 1886. Three years later, after receiving the doctor's degree, he became professor of plant anatomy and physiology in the University of Kharkov. In 1897 he was appointed to a professorship in the University of Warsaw and was made director of the Pomological Garden of Warsaw. He was called to the University of Petrograd in 1900, as professor of plant physiology, where he remained until 1917. In the last-named year Palladin removed to the Crimea, giving lectures in the newly founded university at Simferopol. Later he became director of the Nikitskii Botanical Garden at Jalta.

He was elected to the Russian Academy of Sciences in 1906, and took active part in the work of the Academy, publishing many papers in its proceedings. Election as academician is the highest honor conferred on Russian scientists, and only a few receive this mark of great distinction.

[1] This note is mainly based on a biographical sketch of Palladin, by Professor N. I. Kuznetzov, in the ninth Russian edition of the Physiology. I have been helped in its preparation by Dr. Selman A. Waksman, of the New Jersey Agricultural Experiment Station, and by Mr. L. J. Pessin, of the Mississippi Agricultural and Mechanical College, as well as by Mr. D. N. Borodin, of the New York Office of the Russian Bureau of Applied Botany and Prof. N. Ivanov, of the University of Petrograd.—B. E. L.

Palladin's lectures were always precise and unusually clear. His text-books —on plant anatomy, plant physiology, and systematic botany—show his excellent style of presentation. In his teaching positions Palladin always attracted a group of enthusiastic students. He was a calm and polished leader, always pleasant to work with, who would not quarrel over unessential matters but who understood how to lead the advance persistently toward the finer and greater things. The remarkable precision of his scientific thinking, together with his indefatigable application, placed him at the head of a school of plant physiology that extends far beyond the boundaries of Russia.

Although he was interested in and contributed to many different lines of botanical study, Palladin's main research publications were, from the time of his master's-degree dissertation at Moscow, devoted to the fundamental phenomena of respiration. His many papers on this subject—and those that appeared under joint authorship, with one or more of his colleagues or students —were not confined to the Russian language, and Palladin's name became familiar to readers of the leading French and German journals devoted to botany and to physiological chemistry. To the scientific world at large, as well as to plant physiologists of all nations Palladin's thorough elucidation of some of the most fundamental and baffling aspects of the respiration process will stand as his greatest achievement. Step by step, he and his followers gradually built up a new and clear picture of the chemistry of respiration as it apparently occurs in all living cells.

The main points of the Palladin theory of respiration are somewhat as follows: Under the influence of enzymes, carbohydrates and similar substances are anaerobically decomposed into carbon dioxide and incompletely oxidized organic compounds, these partial oxidations occurring partly at the expense of oxygen derived from the decomposition of water. The hydrogen produced by aqueous decomposition may sometimes be set free, or it may disappear in the reduction of some of the incompletely oxidized compounds just mentioned, but it is regularly oxidized in aerobic respiration, with the formation of water. The aerobic oxidation of hydrogen occurs by two stages: (1) This element combines with respiration pigments (acceptors of hydrogen), thus forming respiration chromogens. (2) The chromogens, in turn, are oxidized by free oxygen, under the influence of oxidizing enzymes, forming water and respiration pigments. Thus, in normal, or aerobic, respiration, the carbon dioxide produced is a product of anaerobic respiration (fermentation), while the water produced is a product of the oxidation, by free oxygen, of anaerobically produced hydrogen. Anaerobic respiration occurs in all living cells, of animals as well as plants, while aerobic respiration is confined to those forms that are supplied with free oxygen and possess adequate oxidizing enzymes. This theory, with all the details that it implies, must be regarded as one of the most brilliant achievements of physiological science, and it may be said to represent the main contribution Palladin made to the advance of appreciative human

knowledge. It is remarkable that the great scholar was able to bring this phase of his studies to such a logical completeness within his lifetime.

Palladin's inspiration still works in the minds and lives of his students, and his contributions to science have become a permanent part of the mental equipment of mankind. The results of his studies and the bent and trend of his clear thought have left a lasting effect, even upon dwellers in far countries. The publication of this second printing of the English edition of the Physiology furnishes a significant illustration of the unity of science, in space as well as in time, and of the true immortality of the scientific spirit.

BURTON E. LIVINGSTON.

DESERT LABORATORY,
TUCSON, ARIZONA,
August, 15, 1922.

AUTHOR'S PREFACE TO THE GERMAN EDITION

This text-book constitutes an improved and enlarged translation of the sixth Russian edition of my Plant Physiology. There are already several excellent text-books on this subject in German, but I venture to hope that the present volume will not be without worth, especially on account of the attention here given to the chemical aspect of physiological processes, and also because of certain peculiarities in the presentation of the subject-matter itself.

It is my pleasant duty to express my hearty thanks to Professor E. Abderhalden, through whose friendly offices the publication of this edition was undertaken. For the translation of the book, my thanks are due to Messrs. Nicolai von Adelung, S. Kostytschew, Georg Ritter and O. Walther, and I am also indebted to the last three gentlemen for valuable advice.

W. Palladin.

EDITOR'S NOTE TO THE SECOND AMERICAN EDITION

In this, the second American edition of Palladin's book, a few typographical and other errors that have come to the editor's attention have been corrected. In a very few cases the wording of the text has been somewhat improved, especially where the old wording was not quite clear. Several new notes by the editor have been added, notably in Part II, Chapters V and VI. While a small number of additional references to the literature have been inserted, no attempt has generally been made to enlarge the scope or increase the number of the citations. The text and notes are generally the same as in the first Edition. The editor is glad to acknowledge valuable assistance received from Dr. Sam F. Trelease, and also to express his thanks to those readers who have called his attention to errors occurring in the first Edition. The publishers have assumed the responsibility for the index.

One new feature has been added, in the form of a summary for each chapter. In preparing the summaries it has been attempted to present a succinct but rather complete statement of the main features dealt with in the respective chapters, with the idea that these résumés may be useful to the student, especially in reviewing the subject. It is suggested, also, that the summary of a chapter may be read with profit *before* reading the chapter itself, the summary thus serving as a sort of general background for the more detailed information gained by perusing the fuller presentation. In some cases new material has been introduced into the summaries, mainly to clear up a few vague transitions from one topic to another that occur in the text, and generally to help the student gain a logical and consistent view-point for the subject as a whole. The editor is alone responsible for the summaries.

The Desert Laboratory,
Tucson, Arizona,
July 15, 1922.

EDITOR'S NOTE TO THE FIRST EDITION

The German edition of this book has gained many friends in institutions where plant physiology is taught and has supplied a need for elementary students not otherwise met. Its small size, together with its generally excellent arrangement and manner of presentation render it very well suited to the use of beginning students who really desire to obtain a general grasp of the subject in a comparatively short time. Its brevity, its conciseness and the readableness of its story are its first attractions, but a further examination reveals the facts that Palladin has been exceptionally thorough in much of his treatment, and that a wealth of well-chosen citations from the literature of plant physiology places in the reader's hands a ready guide to original sources. In the latter regard the text-books originating in our own language are usually deficient, thereby depriving the student of one of his most important rights at the very start—the right to appreciate that the key to the science he is entering really lies in its literature, contributed to by many hundreds of serious workers writing in many languages.

Palladin approaches the subject from the point of view of a student of physiological chemistry, and it is the chemical aspects of plant physiology that here receive greatest emphasis. Most workers in the science will doubtless agree that this is an excellent method of approach. One who has read the book understandingly should be able to plan his own further development, with the aid of the current journals and other contributions, and he will hardly miss the main general idea of present-day physiology, that the future of the subject must rest largely in the development and application of the technique and methods of thinking that characterize the more fundamental sciences of chemistry and physics.

If the German translation has proved to be well suited to the use of serious elementary students, it follows that they should make use of it. Here, however, lies a difficulty. It appears to be the present fashion for graduates of American colleges to be able to really read only the English language, so that the drudgery of virtually digging their way through a German text militates strongly against their becoming familiar with the subject-matter involved; they are apt to fail to grasp the ideas because of a sort of blind struggle to understand the language. This being all too commonly the case, those who take up plant physiology or its applications need, especially, just such a short and scientific treatise as Palladin's book offers, but they need it in their own language, so that they may revert to it now and again without distraction. In this way the student's physiological habits of thought may continue to advance steadily while he is learning to read the foreign tongues that will be requisite for his future work. It was to fill this sort of need among students aiming to make some branch of plant physiology their specialty that an English translation of the German edition was originally

undertaken by Miss Aleita Hopping, working in this Laboratory. Out of her translation the present book has developed.

Aside from its usefulness to university students, Palladin's treatise ought to be of great value to more advanced investigators, especially as it furnishes a summary of a large amount of the literature of the subject, and it is hoped that the present edition may prove helpful to the many English-speaking workers who are engaged in physiological research as applied to agriculture and forestry. To specialists in its own field the book may serve as a convenient means of approach to Palladin's general interpretations. Finally, the numerous Russian references may help to open the domain of Russian science to English-speaking students and to emphasize the rapidly growing importance of Russian research in this subject.

As this translation was nearing completion, Prof. Palladin very kindly furnished the editor with a copy of the seventh Russian edition, with those passages marked in which the latter differs from the sixth Russian edition (from which the German was directly derived), and it seemed desirable to make the present book conform with the author's latest alterations as far as possible. Dr. E. E. Free, also of this Laboratory, has made the necessary translations from the Russian, following Prof. Palladin's notations, and these alterations are included in the English text as here brought forth.

The body of the text aims to be primarily a true translation of the German edition, and the original forms of expression have been retained in practically all cases where this was at all possible in English. The general attitude of the author is so obviously opposed to teleological reasoning that the non-teleological point of view has been made unmistakable in those few places where the German text might leave the reader uncertain in this regard. Palladin's writing is more free from teleological misinterpretations of the relations between conditions and results than is that in most of the text-books hitherto available, and this fact was one of the reasons for the undertaking of the present translation. It will doubtless be a long time before teleology may be deleted from physiological writing and thinking, but readers with a teleological point of view, who may still be satisfied with the consideration of results or effects, in place of conditions or causes that may be as yet unknown, will perhaps not object seriously to an emphasis upon the conviction that permanent progress does not lie in this direction. Few other alterations have been made, these consisting mainly in some modifications in the order of presentation, some slight additions that render certain statements more easily understood, and a very few changes in terminology that seemed desirable. Slight additions are sometimes indicated by being enclosed in brackets.

Editorial notes have been added here and there, in the form of footnotes, which are uniformly signed "*Ed.*" Footnotes not thus designated are Palladin's own. The editorial notes give such additional matter as has seemed desirable, either for completeness of presentation or for a better understanding by English-speaking readers. They constitute, in the aggregate, only a small portion of the volume.

Palladin's treatment of the topics *Growth*, *Movement* and *Reproduction* (which make up the subject matter of Part II) is much less complete than is his treatment of *Nutrition* (Part I), and no attempt has been made by the editor to alter this characteristic of the book. The reader will appreciate the fact that there is available an enormous wealth of knowledge not seriously touched upon in Part II, which he will be able to approach through such other treatises as are mentioned in the list of books that follows this note.

The entire manuscript has been read and criticised by Dr. H. E. Pulling, of this Laboratory, who has contributed much valuable advice in regard to some of the editorial additions.

Since literature references are of prime importance in a book of this kind, and since the citations are not always clearly, fully, nor uniformly given, either in the German or in the Russian, it became necessary to verify these and correct them when necessary. This arduous task has been carried out by Mrs. Grace J. Livingston. Nearly all of the references have thus been verified, and the form of citation has been rendered uniform, as far as possible, throughout the work. Dr. Free has cared for the Russian citations. No attempt has been made to indicate what portions of any of the citations are due to correction or completion. Citations that it has been impossible to verify are given just as they appear in the German (or Russian), and are followed by an asterisk(*) to signify this. Some additional literature references have been inserted by the editor, these being generally enclosed in brackets, unless they occur in editorial notes.

The rapidly increasing frequency of references to Russian authors in scientific literature is accompanied by much discrepancy in the English spelling of Russian proper names. This matter will require more serious attention from scholarly scientific writers in the future than has been accorded it in the past, and an attempt is here made at least to avoid the exacerbation of a condition that is already bad enough. The difficulty has perhaps arisen mainly through the fact that our acquaintance with Russian science is almost wholly based on writings in other foreign languages, especially in French and German. We have too frequently taken the German or French transliteration, as the case may be, without regard to the fact that this almost always leads to mispronunciation by the English reader. Thus, Pavlov often appears as *Pawlow*, which is as incorrect in English as it is correct in German. The name of the author of the present volume furnishes another example; we have *W. Palladin* where we should have V. Palladin. (In this particular case, the silent final *e* of the Russian and of the French form of this name should be dropped in English, to avoid the resulting lengthening of the last syllable and even the misplacing of the accent, which is penultimate. The name is pronounced Pal-lad′-in,[1] like Aladdin.)

In those cases where it is quite clear that a proper name ought to be regarded as Russian, an English spelling is here adopted that will lead to no serious ambiguity as to pronunciation and that can be readily retransformed into the Rus-

[1] This is authoritative, from Professor Palladin himself.

sian. In these transliterations of Russian words into English the rules of the U. S. Library of Congress have been followed, with a few slight modifications, as follows: *i͡a*, *i͡u*, *i͡e* are all given as *ia*, *iu*, *ie*; *i*, *ï* and *ĭ* are all given as *i*; the sign of the silent letter between two others (′) is omitted (Krasnoselskaia is used instead of *Krasnosel′skaia*) and Yegunov is employed instead of *Egunov*, to insure proper pronunciation. When the name is not certainly Russian and when several spellings occur, the commonest form occurring in the German book is adopted. In those cases where the paper cited is in Russian the author's name is transliterated into English in the citation, as well as in the text, the title of the paper being translated into English unless a title in French or German is available. In citations from languages other than Russian, author's names are given just as they occur in the publications cited. The two or three spellings that thus occur for the same Russian name are all given in the index, with the requisite cross-references. Thus, references to Ivanov are all given under this spelling, but *Ivanoff* and *Iwanow* are also given, with the notation, "see Ivanov."

The index is somewhat more comprehensive than is the case with the original, and authors' names have been inserted in the same alphabet with the names of subjects. This feature of the index amounts practically to a bibliography; references are given to all pages where the name in question is mentioned, and those pages that bear footnote citations of this name are indicated by full-face type.

A note on the form of citation employed in this volume, and a selected list of books bearing on plant physiology, are added after the present note. It is hoped that these additions, as well as the citations of the book itself, may prove serviceable to those who wish to acquire familiarity with the far-flung literature of a subject that embraces the principles of many separately named sciences, that brings into a single narrative such topics as ionization, adsorption, photosynthesis, fermentation, the forcing of azalias and the keeping-qualities of apples.

LABORATORY OF PLANT PHYSIOLOGY
OF THE JOHNS HOPKINS UNIVERSITY.

FORM OF CITATION

The form of citation employed in the footnotes uses (1) an Italic Roman numeral (followed by a comma) for the series number, (2) a black-face Arabic numeral (followed by a colon) for the volume number, (3) a superscript numeral for a subdivision of the volume, (4) Arabic numerals, in ordinary type, for the first and last page of the article cited (separated by a dash, and the second number followed by a period), and (5) an ordinary Arabic numeral for the year of publication (followed by a period). When several pairs of page numbers are given, as when an article is continued through several issues of the serial, these pairs are separated by commas. Where there is no volume number the volume has to be designated by its year number, and this is given in the place that would be occupied by the volume number, and in black-face type. Sometimes this year number, for which the volume stands, is not the same as the year of publication. In cases where a volume extends into more than one year, the year of publication of the volume frequently gives place to two year numbers (separated by a dash). When adequate information was available a single year number is given in the cases just mentioned, referring to the year of publication of the article cited rather than to the two or more years of the volume as a whole.

Author's names are given in black-face type, the surname preceding the initials or given name **Idem** (black-face type) denotes a repetition of the author's name, or of the authors' names, next preceding. *Ibid.* (Italics) denotes repetition of the name of the serial next preceding.

The rather customary promiscuous scattering of capital letters through citations has been avoided; words or their abbreviations begin with capital letters only (1) when they are considered as beginning a sentence, (2) when they are proper names, (3) when they *begin* the proper name of a serial (as, *Bot. gaz.*, *Plant world*), (4) when they are important words in the proper name of a society, institution, etc. (as, *Roy. Soc. London*, *Missouri Bot. Gard.*), or (5) when they are German nouns (compare *Ann. bot.*, *Compt. rend.*, *Bot. Zeitsch.*, *Jahrb. wiss. Bot.*). The abbreviations employed for the names of serials appearing in the citations are, it is hoped, self-explanatory.

When a citation appears more than once, it is given in full only in the first instance, and later occurrences include simply the author's name, the year, and (in brackets) a reference to the page of this book where the full citation may be found.

A CLASSIFIED LIST OF BOOKS FOR REFERENCE IN PLANT PHYSIOLOGY

Physics, General Chemistry and Mathematics

Bernthsen, A., A Text-book of Organic Chemistry, English translation, edited by J. J. Sudborough. 674 p. New York, 1907.

Comstock, Daniel F., and Troland, Leonard T., The Nature of Matter and Electricity, an Outline of Modern Views. 203 p. New York, 1917.

Davenport, C. B., Statistical Methods, with Special Reference to Biological Variation. 3d ed. 225 p. New York, 1914.

Holleman, A. F., and Cooper, H. C., A Text-book of Inorganic Chemistry. 6th Eng. ed. 527 p. Philadelphia, 1921.

Holleman, A. F., and Walker, A. J., A Text-book of Organic Chemistry. 5th Eng. ed. 642 p. New York, 1920.

Mellor, J. W., Higher Mathematics for Students of Chemistry and Physics, with Special Reference to Practical Work. 641 p. London, 1909.

Northrup, E. F., Laws of Physical Science, a Reference Book. 210 p. Philadelphia, 1917.

Nutting, P. G., Outlines of Applied Optics. 234 p. Philadelphia, 1912.

Ostwald, Wilhelm, The Principles of Inorganic Chemistry. Translated by Alexander Findlay. 3d ed. 801 p. London, 1908.

———, The Fundamental Principles of Chemistry. Translated by Harry W. Morse. 349 p. New York, 1909.

———, Introduction to Chemistry. Translated by William T. Hall and Robert S. Williams. 368 p. New York, 1911.

Willows, R. S., and Hatschek, E., Surface Tension and Surface Energy and their Influence on Chemical Phenomena. 114 p. 2d ed. London, 1919.

Physical Chemistry and Colloid Chemistry

Clark, W. M., The Determination of Hydrogen Ions. 2d ed. 480 p. Baltimore, 1922.

Cohen, Ernst, Physical Chemistry for Physicians and Biologists. Translated by Martin Fischer. 343 p. New York, 1903.

Findlay, Alexander, Osmotic Pressure. 84 p. London, 1913.

Freundlich, Herbert, Kapillarchemie, eine Darstellung der Chemie der Kolloide und verwandter Gebiete. 591 p. Leipzig, 1909.

Hatschek, E., An Introduction to the Physics and Chemistry of Colloids. 172 p. 4th (revised) ed. London, 1922.

Jellinek, Karl, Lehrbuch der physikalischen Chemie. Vol. I, 715 p. Stuttgart, 1914. Vol. II, 909 p. Stuttgart, 1915. [Two more volumes to follow.]

Lewis, William C. McC., A System of Physical Chemistry. 2d ed. 3 vols. 494, 403 and 209 p. London and New York, 1918, 1919, 1921.

Nernst, Walther, Theoretical Chemistry from the Standpoint of Avogadro's Rule and Thermodynamics. Translated by Chas. Skeele Palmer. 697 p. London and New York, 1895.

Ostwald, Wolfgang, A Handbook of Colloid Chemistry. 2d Eng. ed., translated from the 3d Ger. ed. by Martin Fischer, with notes by Emil Hatschek. 266 p. Philadelphia, 1919.

———, An Introduction to Theoretical and Applied Colloid Chemistry, "The World of Neglected Dimensions." Translation by Martin H. Fischer. 232 p. New York, 1917.

———, Die Welt der Vernachlässigten Dimensionen. 219 p. Dresden and Leipzig, 1915.

Philip, J. C., Physical Chemistry, Its Bearing on Biology and Medicine. 326 p. London, 1920.

Taylor, W. W., The Chemistry of Colloids and Some Technical Applications. 3d impression. 328 p. London, 1918.

van't Hoff, J. H., Lectures on Theoretical and Physical Chemistry. Translated by R. A. Lehfeldt. Part I, Chemical Dynamics. 254 p. Part II, Chemical Statics. 156 p. Part III, Relations Between Properties and Composition. 143 p. London, 1898, 1899, and 1900.

Washburn, Edward W., An Introduction to the Principles of Physical Chemistry from the Standpoint of Modern Atomistics and Thermodynamics. 2d ed. 516 p. New York, 1921.

Zsigmondy, Richard, Kolloidchemie. 281 p. Leipzig, 1912.

Zsigmondy, Richard, Spear, Ellwood B., and Norton, John Foote, The Chemistry of Colloids. [Part I is an English translation of Zsigmondy's Kolloidchemie, translated by Spear. Part II consists of Industrial Colloid Chemistry (by Spear) and a chapter on Colloidal Chemistry and Sanitation (by Norton).] 288 p. New York, 1917.

Soil Science and Climatology

Cameron, F. K., The Soil Solution, the Nutrient Medium for Plant Growth. 136 p. Easton, Pa., 1911.

Clements, F. E., Aeration and Air-content. the Role of Oxygen in Root Activity. Carnegie Inst. Wash. Publ. No. 315. 183 p. 1921.

Ehrenberg, Paul, Die Bodenkolloide. 563 p. Dresden and Leipzig, 1915.

Hall, A. D., The Soil, an Introduction to the Scientific Study of the Growth of Crops. 3d ed. 352 p. London, 1920.

Hann, Julius, Handbuch der Klimatologie. 3 vols. 394, 426 and 713 p. Stuttgart, 1908–11.

———, Handbook of Climatology. Part I, General Climatology. Translated from 2d Ger. ed., with additional references and notes, by Robert De Courcy Ward. 437 p. New York and London, 1903.

Hilgard, E. W., Soils, Their Formation, Properties, Composition, and Relations to Climate and Plant Growth. 593 p. New York, 1906.

Mitscherlich, Eilh. Alfred, Bodenkunde für Land und Forstwirte. 2te Aufl. 317 p. Berlin, 1913.

Russell, Edward J., Soil Conditions and Plant Growth. 4th ed. 406 p. London and New York, 1921.

Ward, Robert De Courcy, Climate, Considered Especially in Relation to Man. 372 p. New York, 1908.

Warrington, Robert, Lectures on Some of the Physical Properties of Soil. 231 p. Oxford, 1900.

General Physiology, Physiological Chemistry and Physiological Physics

Abderhalden, Emil, Handbuch der biochemischen Arbeitsmethoden. 9 vols. Berlin, 1910–19.

———, Biochemisches Handlexikon. Vols. 1–7, Berlin, 1911. Vol. 8, 1913; vol. 9, 1915. [Includes very extensive literature references.]

Bayliss, William Maddock, Principles of General Physiology. 3d ed. 862 p. London and New York, 1920. Includes an extensive bibliography.

Czapek, Friedrich, Biochemie der Pflanzen. 1te Aufl. 2 vols. Jena, 1905. [Includes very extensive citations of the literature.] 2te Aufl. 3 vol., (828 p.). Jena, 1913. [Only first vol. has appeared.]

Effront, Jean, Enzymes and Their Applications. Translated by Samuel C. Prescott. 322 p. New York, 1902.

Euler, H., General Chemistry of the Enzymes. Translated by T. H. Pope. 323 p. New York, 1912.

———, Grundlagen und Ergebnisse der Pflanzenchemie, nach der Schwedischen Ausgabe bearbeitet. I Teil, Das chemische Material der Pflanzen. 239 p. Braunschweig, 1908. II Teil, Die allgemeinen Gesetze des Pflanzenlebens. III Teil, Die chemischen Vorgänge im Pflanzenkörper. The last 2 parts in one vol. 298 p. Braunschweig, 1909.

Haas, P., and Hill, T. G., An Introduction to the Chemistry of Plant Products. 3d ed. 414 p. London, 1921.

Henry, Thomas Anderson, The Plant Alkaloids. 466 p. Philadelphia, 1913.

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Loeb, J., The Dynamics of Living Matter. 233 p. New York, 1906.

———, The Mechanistic Conception of Life: Biological essays. 232 p. Chicago, 1912.

———, The Organism as a Whole, from the Physicochemical Viewpoint. 379 p. New York and London, 1916.

Mathews, Albert P., Physiological Chemistry. 3d ed. 1154 p. New York, 1920.

McClendon, J. F., Physical Chemistry of Vital Phenomena, for Students and Investigators in the Biological and Medical Sciences. 240 p. Princeton, 1917. [Includes an extensive bibliography.]

Onslow, M. W., Practical Plant Biochemistry. 178 p. Cambridge, 1920.

Pütter, August, Vergleichende Physiologie. 721 p. Jena, 1911.

Verworn, Max, Allgemeine Physiologie, ein Grundriss der Lehre vom Leben. 6 ed. 766 p. Jena, 1915.

———, General Physiology, an Outline of the Science of Life. Translated from the 2d German edition by F. S. Lee. 599 p. London, 1899.

Plant Morphology and General Botany

Chamberlain, C. J., Methods in Plant Histology. 3d ed. 314 p. Chicago, 1915.

De Bary, Heinrich Anton, Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Translated and annotated by F. O. Bower and D. H. Scott. 659 p. Oxford, 1884.

Ganong, Wm. F., A Text-book of Botany for Colleges. 604 p. New York, 1917.

Haberlandt, G., Physiological Plant Anatomy. Translated by M. Drummond. 777 p. London, 1914.

Jordan, Edwin O., A Text-book of General Bacteriology. 7th ed. 744 p. Philadelphia and London, 1921.

Martin, J. N., Botany with Agricultural Applications. 2d ed. 604 p. New York, 1920.

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Palladin, W. I. [V. I.], Pflanzenanatomie. Nach der 5ten Russischen Aufl., übersetzt und bearbeitet von S. Tschulok. 195 p. Leipzig and Berlin, 1914.

Zimmermann, A., Botanical microtechnique. Translated by J. E. Humphrey. 296 p. New York, 1893.

Schimper, A. F. W., Plant Geography Upon a Physiological Basis. Translated by W. R. Fischer. 839 p. Oxford, 1903.

Stevens, W. C., Plant Anatomy from the Standpoint of the Development and Functions of the Tissues, and Handbook of Microtechnic. 3d ed., 399 p. Philadelphia, 1916.

Plant Physiology

Atkins, W. R. G., Some Recent Researches in Plant Physiology. 328 p. London and New York, 1916.

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INTRODUCTION

> La physiologie est une des sciences les plu dignes de l'attention des esprits élevés par l'importance des questions, qu'elle traite, et de toute la sympathie des hommes de progrès par l'influence, qu'elle est destinée à exercer sur le bienêtre de l humanité.
>
> —*Claude Bernard.*

The aim of plant physiology is to gain a complete and thorough knowledge of all the phenomena occurring in plants, to analyze the complex life processes so as to interpret them in terms of simpler ones and to reduce them finally to the principles of physics and chemistry. It is evident from this statement that physiology is dependent upon physics and chemistry, and that progress in physiology depends, in great measure, upon progress in these two other sciences. Only since the end of the eighteenth century, when the principle of the conservation of mass was formulated by Lavoisier, and chemistry became an exact science, did it become possible for physiology also to begin to assume this character. Since that time it has been possible to employ the balance in precise studies of the materials that enter and leave plants. The well-known experiment of van Helmont (1577–1644), performed long before those of Lavoisier, may be cited as an early though but partially successful attempt to use the balance for determining the source of the materials found in the plant body. A willow branch weighing 5 pounds was potted in 200 pounds of dry soil and watered with rain-water. After five years the weight of the rooted branch was estimated to be 164 pounds, while the dried earth showed a loss in weight of only 2 ounces. Van Helmont concluded from this that the material of the plant was formed from water, but this inference is incorrect, since the surrounding air was not considered. He would have been justified in concluding, however, that the greater part of the non-aqueous material of plants *does not come from the soil.*

Besides the discoveries of Lavoisier, another important event in the history of chemistry must be alluded to here, the synthesis of urea, accomplished by Wöhler in 1828. Up to that time organic compounds had been obtained only from living organisms, and the idea prevailed that the synthetic preparation of such compounds from inorganic materials was impossible and that their formation presupposed the participation of a special vital activity. Wöhler's discovery, together with subsequently successful syntheses of various other organic compounds, have shown that no vital force is essential to the formation of such substances.

The organic and inorganic compounds of carbon are often combined in a single group, but there is an essential difference between them for the physiologist; all organic substances contain a store of energy, since they give off heat

when burned, while the inorganic carbon compounds cannot be burned. The heat of combustion, measured in calories, serves as an index of the energy content of organic compounds. By a large calory, or kilogram-calory (Cal., or kg.-cal.) is meant the amount of heat necessary to raise the temperature of 1000 g. of water from 0° to 1°C.; by a small calory, or gram-calory (cal. or g.-cal.) is meant the amount of heat necessary to raise the temperature of 1 g. of water the same amount.[a]

The following table shows the amounts of heat obtained from the combustion of 1 g. of various substances, expressed in kilogram-calories.

Hydrogen	34.6
Carbon	8.0
Linseed oil	9.3
Ethyl alcohol (C_2H_6O)	7.1
Gluten flour	5.9
Ammonia (NH_3)	5.3
Starch ($C_6H_{10}O_5$)	4.1
Glucose ($C_6G_{12}O_6$)	3.7
Asparagin ($C_4H_2N_2O_3$)	3.3

It is evident from this table that hydrogen develops much more heat during combustion than does carbon. The more oxygen the molecule of a substance contains, the less is its heat of combustion, and it is for this reason that ethyl alcohol develops more heat than starch. The introduction of hydrogen into the molecule, on the contrary, produces a great increase in the heat of combustion; thus, oil develops more heat than does pure carbon, while ammonia, without any carbon at all—but because of its high hydrogen content—produces a far greater amount of heat than does either starch or glucose.

Wöhler's discovery led to a great advance in the physico-chemical interpretation of physiological processes. But there were still other difficulties to overcome. Many chemical reactions go on in plants and animals at the temperature of the organism (*i.e.*, about ordinary room temperatures), while the same reactions outside the organism occur only at much higher temperatures or with the aid of strong acids. For instance, as will be seen later, plant respiration is a process of oxidation or combustion, but it proceeds at medium temperatures, while ordinary combustion requires a very high temperature. While plant and animal substances outside of the organism generally undergo oxidation slowly at ordinary temperatures, with the oxygen of the air, they are oxidized much more rapidly in the organism, at the same temperatures. This discrepancy was explained by the theory of catalysis, advanced by Berzelius in 1836. Catalytic action, according to this author, is a process wherein certain substances (called catalyzers) are capable of accelerating chemical reactions between other substances, by the presence of the catalyzer alone, independently

[a] The gram-calory is frequently defined as the heat required to raise the temperature of a gram of water one degree Centigrade, but this is not precise, since the specific heat and the heat of vaporization of water vary with its temperature. The definition given in the text is that of the 0-degree gram-calory. Other calories are in use, as the 15-degree gram-calory, the heat needed to alter the temperature of a gram of water from 14.5° to 15.5°C., etc.—*Ed.*

of its chemical affinities and without its being used up in the reaction. A substance is regarded as a catalyzer if it alters the velocity of a chemical reaction without itself appearing in the end-products. For instance, if a weak solution of sulphuric acid is allowed to act upon metallic zinc, the evolution of hydrogen is very slow if both reagents are very pure, but the addition of a few drops of platinic chloride is sufficient to cause a stormy evolution of the gas. The reaction proceeds, either in the presence or in the absence of the platinum salt, according to the equation,

$$Zn + H_2SO_4 = ZnSO_4 + H_2.$$

The platinic salt does not enter into the reaction and so acts simply as a catalyzer.

Various kinds of catalyzers have now been shown to exist in plants and animals, and these are called ferments[b] or *enzymes*. Enzymes, according to Wilhelm Ostwald, are catalyzers formed in the organism during the life of the cell, and it is with their help that the living organism effects most of its chemical processes. Not only are digestion and assimilation regulated entirely by enzymes, but the production of chemical energy by oxidation, at the expense of the oxygen of the air—a process forming the basis for the life activity of most organisms—is also made possible and directed by these catalyzers. It is well known that oxygen is a very inactive substance at the temperature of organisms and that the maintenance of the life process would be impossible without an acceleration of chemical reaction velocities. In plants special enzymes (oxydases) are indeed found that act, either within or without the organism, to produce the oxidation of various substances at room temperature.

The attention of scientists was especially attracted by the enzymes of lower plants, such as yeasts and bacteria, these plants having been themselves designated as "organized ferments." The most important discoveries in the physiology of yeasts and bacteria are due to Pasteur,[c] who proved the absence of spontaneous generation in the lower organisms, developed a clear conception of the various kinds of fermentation, and devised perfect methods for the control of infectious diseases. The worker in the shop, as well as the farmer in the field, the physician at the bedside, the veterinarian treating domestic animals, the brewer handling his yeast, are all now guided by the ideas of Pasteur.

A physical discovery that was very important to physiology must here be mentioned, the formulation of the principle of the conservation of energy, by Julius Robert Mayer, in 1840. Mayer demonstrated that no energy is lost in

[b] The noun ferment should be dropped, as unnecessary and apt to be misleading. What were once called unorganized ferments are enzymes, and organized ferments (such as yeasts, bacteria, etc.) may be called by name or referred to as fermentation organisms. The word enzyme is frequently mispronounced; it should be pronounced as if spelled *enzim*, with the first vowel accented and the second short. The spelling enzym is better, but has not yet come into general use in English.—*Ed.*

[c] Students of chemical physiology should be well acquainted with Pasteur's life and work. See: **Vallery-Radot, René,** The Life of Pasteur. Translated by Mrs. R. L. Devonshire. ix + 484 p. New York, 1915.—*Ed.*

the various chemical reactions, but that it is transformed from the potential into the kinetic condition, or *vice versa*. In the combustion of coal, for example, heat is liberated, while by the reverse process, the decomposition of carbon dioxide, heat is stored. Since combustibility is a characteristic of all organic compounds, their formation from carbonic acid must therefore be accompanied by an intake of heat and a storing of potential energy, which may be subsequently liberated during combustion. In all investigations concerning the transformations of materials in plants it must be clearly stated whether energy is stored or released, since only thus can it be clear what is the meaning and importance of such transformations in the general activity of the organism.

At first glance, some phenomena seem to present exceptions to the principle of the conservation of energy and to exhibit no quantitative relation between cause and effect. For example, a small spark may cause the explosion of an enormous amount of gunpowder and thus produce tremendous destruction. It might seem here that a small cause has entailed a great effect; in reality, however, the same amount of energy was liberated in the explosion as was originally present—in a potential form—in the gunpowder. The spark served only to initiate the change of this energy from one condition to the other. A small concussion of the air is often sufficient to cause the fall of a huge boulder from a great height, but the work thereby performed is exactly equal to the amount necessary to replace the boulder in its original position. The pressure of the air serves here as the trigger that produces the discharge.

In considering the great importance of enzymes in the chemical processes of plants it must be realized that their part in the various reactions does not consist in a simple release. Bredig was quite right when he said, "We still find much vagueness in the text-books as to whether, in this matter of the contact action of substances such as acids and enzymes in the hydrolysis of esters, carbohydrates, glucosides, etc., we have to do with the initiation of a reaction incapable of occurring by itself, or only with the *acceleration* of a reaction that takes place so slowly (in the absence of the catalyzer) as to be almost imperceptible, but that is nevertheless already in operation. The question is, therefore, to use a mechanical figure, whether the enzyme sets into operation a machine previously held at rest by a trigger-pin, or whether the enzyme serves only as a lubricant to hasten the action of the machine (the chemical reaction), which would otherwise be very slow and almost imperceptible, because of great resistance."[1] Enzymes accelerate reactions that would otherwise progress but slowly (Wilh. Ostwald) and they are thus comparable only to the "lubricant."[d] On the other hand, the touch that causes a reaction-movement of the leaves of *Mimosa pudica* (the sensitive plant) may be regarded as a typical example of a discharge or release.

The causes that produce certain phenomena and the conditions that first render them possible must also be differentiated. For instance, if solid calcium

[1] **Bredig, G.**, Die Elemente der chemischen Kinetik, mit besonderer Berücksichtigung der Katalyse und der Fermentwirkung, Ergeb. Physiol. 1: 134–212. 1902.

[d] Enzymes frequently appear to alter the end-point of a reaction, so that it proceeds farther in their presence than without them.—*Ed.*

sulphate is mixed with solid barium chloride there is no reaction; when water is added, however, barium sulphate and calcium chloride are formed. This reaction is caused by the chemical attraction of the elements, the water acting only as a necessary condition. Thus releases, which are conditioning factors, must be distinguished from real causes.[e]

Plants have an internal structure, being composed of cells of various forms and sizes. The life of an organism is the sum-total of the life activities of the individual cells composing it, and the study of plant physiology presupposes an acquaintance with the internal structure or anatomy of the plant. Familiarity with the miscroscope is essential in physiological study, since many important physiological questions can be solved by its use.

For the study of many physiological phenomena—those of growth and enlargement, for example—a knowledge of the structure of the given plant and an acquaintance with the external conditions affecting it, are not sufficient; it must also be remembered that the plant has developed from a long series of ancestors whose form and mode of living have not been without effect upon the offspring. In these cases, therefore, heredity must be taken into account.[f]

[e] The definition of the term *cause* involves difficulties. It is probably best to consider that all changes are determined (in quantity, rate and direction) by a set of controlling conditions, the cause—in the ordinary sense—being simply the last one of these necessary conditions to be fulfilled. For a discussion of this matter see: **Verworn, Max,** Kausale und Konditionale Weltanschauung, Jena, 1912.—*Ed.*

[f] This is somewhat vague; the phenomena in question are assuredly conditioned at any given time by the internal and external conditions then prevailing. The nature of the ancestors of a plant and the surroundings under which these lived are but secondary conditions, which have been influential in determining what are the present internal conditions (what the plant *is* now), but which are, in themselves, without any present direct influence upon its processes. The phenomena connoted by the term heredity have played an important rôle in determining the present internal conditions, and these latter, together with the present surroundings, are now influential in the determination of physiological phenomena.—*Ed.*

PART I

PHYSIOLOGY OF NUTRITION

CHAPTER I

ASSIMILATION OF CARBON AND OF THE RADIANT ENERGY OF THE SUN BY GREEN PLANTS

§1. **Importance of the Assimilation of Carbon by Green Plants.**—Plants may be classified according to their color into two groups, those that are green and those that are not. The green color forms such a conspicuous characteristic of many plants that certain ones are sometimes spoken of as "greens." The general distribution of the green coloring would itself suggest that some important property must be connected with it, and such is indeed the fact; upon this green coloring depends one of the main cosmic functions of plants, the building up of organic compounds from inorganic substances. A simple experiment will show this. A seed is placed in quartz sand and is watered from time to time with a solution of mineral salts. A plant grows from the seed, blooms and bears fruit. Comparison of the amount of organic material originally present in the seed, with the corresponding amount found in the grown plant, shows that the latter amount is very much greater. If follows that *green plants are able to form organic compounds from inorganic ones.* Animals, and plants without green pigment, generally lack this power; they obtain organic compounds only after these have been already manufactured by green plants. The formation of organic substances by green plants is thus not only important from the standpoint of plant physiology, but it acquires a much broader interest, since the whole animal kingdom, including even mankind, is dependent upon green plants. In a physiological sense, *green plants form the connecting link between the animal and mineral kingdoms.*

Since all organic compounds are characterized by their carbon content and by their combustibility—the latter property implying that energy was stored up in their formation—the study of plant physiology may begin with an inquiry as to the sources of the carbon and the energy necessary for the formation of organic compounds in the organism. The answer is derived mainly from the study of the assimilation of carbon dioxide. This process consists, essentially, in the absorption of carbon dioxide by the green parts of plants and in the elimination of oxygen, in sunlight. Since the volumes of the two gases involved in this process are found to be about equal, it follows that for each molecule of carbon dioxide absorbed a molecule of oxygen is eliminated; $CO_2 = O_2 + C$ (principle of Avogadro). The carbon remains in the plant and thus produces an increase in its weight, this process being a part of what is called nutrition.

Since the formation of carbon dioxide in the combustion of carbon is accompanied by the liberation of heat, energy must be stored in the reverse process, the decomposition of carbon dioxide. From this it is clear why sunlight is so important in this decomposition; the energy of the sunshine absorbed by the plant is partly used in the decomposition of carbon dioxide and in the synthesis of other carbon compounds. The green coloring matter, chlorophyll, serves as a screen which absorbs the sun's rays and makes this energy fixation possible.

§2. **Exchange of Gases.**—Our first knowledge of the elimination of oxygen by green plants was obtained by Priestley,[1] in 1772. Since animals utilize "dephlogisticated air" (as Priestley, its discoverer, called oxygen) and thus render the atmosphere unfit for the maintenance of combustion and respiration, he sought a reverse process by which the air might be improved, and he found this process in plants. He placed plants under a bell-jar of air that had been vitiated by animal respiration and was thus unfit for the maintenance of combustion and respiration, and found that after some time the air became again capable of supporting these processes. Unfortunately, however, subsequent repetition of this experiment did not always give the same result. Sometimes the plants improved the air, often they did not, and Priestley did not know the reason for these variations. It remained for Ingen-Housz[2] to show that the purifying of the air was effected only by the green parts of plants, and only in sunlight. The importance of this process in the life of the plant was still unexplained; it was regarded as a purposeful arrangement for the improvement of the air for animals. Ingen-Housz had no clear idea as to what gas is taken in by the plant, and even thought that the gas given off by metals under the action of acids might be thus improved by plants. Senebier[3] was later able to show that carbon dioxide alone is absorbed, and that this absorption is a nutritive process. De Saussure[4] then found that the volume of oxygen given out was equal to that of carbon dioxide taken in, that the decomposition of the last-named gas was most rapid when one part of it was present in eleven parts of air, and, finally, that an increase in the weight of the plant occurred as a result of this absorption and decomposition. All these questions were finally taken up by Boussingault,[5] in a series of precise experiments. The equality of the volumes of the exchanged gases was established. By an experiment upon the decomposition of carbon dioxide by green plants in a mixture of this gas and hydrogen or nitrogen, Boussingault was able to show that the decomposition in question began immediately after the illumination of the apparatus, and ceased as soon as it was darkened. Phosphorus was used to show the presence of

[1] **Priestley, Joseph,** Experiments and observations on different kinds of airs. 324 p. London, 1774.

[2] **Ingen-Housz, Jan,** Experiments upon vegetables, discovering their great power of purifying common air in the sunshine, and of injuring in the shade and at night. London, 1779. [Ref. in Ger. ed. is apparently to Scherer's translation, 3 v., Vienna, 1786, 1788, 1790. This was from author's French ed., 1780.]

[3] **Senebier, J.,** Mémoires physico-chimiques sur l'influence de la lumière solaire pour modifier les etres des trois règnes de la nature et sur-tout ceux du règne végétal. Genève, 1782. **Idem,** Physiologie végétale. Genève, 1800.

[4] **Saussure, Nicolas Théodore de,** Recherches chimiques sur la végétation. Paris, 1804.

[5] **Boussingault, Jean B. J. D.,** Agronomie, chimie agricole et physiologie. 2nd ed. Paris, 1860–1891.

oxygen, a piece of this substance being exposed in the experiment chamber. As soon as light was allowed to enter the apparatus the formation of a white vapor indicated the presence of oxygen, and when the apparatus was darkened the fumes already formed disappeared and no more appeared, showing that the elimination of oxygen had ceased. [The fumes are suspended phosphorus pentoxide (P_2O_5), which dissolves in water, forming phosphoric acid (H_3PO_4), and thus disappears soon after the apparatus is darkened.]

Since this experiment was performed in a closed chamber with a high carbon dioxide content, it was questionable whether the results obtained might justify the conclusion that plants can utilize the small amount of carbon dioxide in the air under natural conditions (0.028–0.04 per cent.). To clear up this point, Boussingault placed a plant in a jar through which a current of air was passed. Analysis of the entering air and of that passing out showed that the plant was able, under favorable conditions of light, to absorb almost all of the carbon dioxide that entered the jar. Regarding this experiment of Boussingault, Timiriazev says:

> To what degree the precision of this experiment aroused the wonder of his contemporaries (as did most of Boussingault's researches) can best be shown by an anecdote which I heard from Boussingault himself. "The investigation was undertaken jointly with Dumas, with weighings and records independently made by each worker, in order to secure more reliable results. At first all went well, and the plants decomposed carbon dioxide as they were expected to do. Then things suddenly changed. On a bright, sunny day, the plants began to *produce* carbon dioxide instead of decomposing it. In the evening we examined the result with astonishment and stared at each other in blank amazement. Involuntarily we remembered the misfortune that had attended Priestley when he attempted to repeat his famous experiment. Several days passed by. Then, one fine morning, Regnault, the famous physicist, who had been watching our experiment with much interest, began to laugh at our long faces and admitted that he had been to blame for our misfortune. Every day, while we were at luncheon, he had sneaked over to our apparatus and breathed into it, 'in order,' as he explained, 'to be convinced that you were not taking a *u* for an *x*, and could really determine such small amounts of carbon dioxide.' "[1]

De Saussure and Boussingault showed that the ratio $\frac{CO_2}{O_2}$ is generally equal to unity. However, it must be remembered that green plant parts also respire while they are assimilating carbon dioxide; that is, they carry on the reverse process, wherein carbon dioxide is eliminated and oxygen is combined. Although the process of respiration is much weaker than that of photosynthesis (or "carbon assimilation"[a]), still each must be kept distinct and it must be

[1] **Timiriazev, K. A.,** From the field of plant physiology. Public lectures and addresses. [Russian.] Moscow, 1888. P. 245.

[a] The term photosynthesis has now come into very general use among English and French physiologists, in place of the more cumbersome expressions previously employed, and there seems to be little room for doubt that it will eventually become universal. The word is of American origin. Barnes (**Barnes, C. R.,** On the food of green plants. Bot. gaz. **18**: 403–411. 1893) suggested *photosyntax*, and the other and better form is due to McMillan, and its general introduction to MacDougal. Ewart is partly right in the footnote he appended to his translation of Pfeffer's Plant Physiology (1: 302. Oxford, 1900), but his objections do not appear valid as against the use of *photosynthesis*. Of course, this should include all possible forms of chemical synthesis brought about through the action of light, but the formation of

found out how the ratio $\frac{CO_2}{O_2}$ varies, independently of respiration. Bonnier and Mangin[1] investigated this and found the value of the ratio to be really somewhat less than unity. So the plant gives off not only the equivalent of all the oxygen originally contained in the absorbed carbon dioxide, but also a smaller portion of oxygen arising from the water that is decomposed in photosynthesis.[2]

As to methods of investigation, the decomposition of carbon dioxide can be detected in the following manner. A cut leaf is placed in a calibrated glass tube (Fig. 1), the upper end closed and the lower, open end dipping into mercury. Then a part of the air is removed by a rubber tube and the level of the mercury rises. The volume of the remaining air is read, after which some carbon dioxide is admitted from a gasometer and the gas volume is again determined. The apparatus is not placed in light and after some time the gas volume is once more recorded. The remaining carbon dioxide is removed by injecting some concentrated potassium hydroxide solution, and the diminished gas volume is again read; pyrogallol is next introduced, and a final reading, after the removal of oxygen by the pyrogallol, gives the amount of nitrogen that remains. The numbers obtained permit the determination of the amounts of carbon dioxide absorbed and of oxygen liberated.[3]

A less exact method consists in counting the number of gas bubbles

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carbohydrate out of carbon dioxide and water is by far the most important form of photosynthesis, and the term may readily be qualified whenever need arises. Thus, we may distinguish *chlorophyll photosynthesis of carbohydrate* from other photosyntheses. The word assimilation has been employed in so many different senses that to attempt its use as a precise term in this connection here seems unadvisable.—Jörgensen and Stiles prefer, however, to employ the "rather non-committal expression," *carbon assimilation*, and they do so in their recent very excellent monograph on this subject, which should be referred to in connection with this entire chapter, and which should become familiar to every student of plant physiology. See: **Jörgensen, Ingvar,** and **Stiles, Walter,** Carbon assimilation, a review of recent work on the pigments of the green leaf and the processes connected with them. New phytologist reprint No. 10. London, 1917. (This is reprinted from a series of articles having same title, in New phytol. **14–16.** 1915–17.)—*Ed.*

[1] **Bonnier, Gaston,** and **Mangin, Louis,** L'action chlorophyllienne séparée de la respiration. Ann. sci. nat. Bot. *VII*, **3**: 5–44. 1886.

[2] It will be seen later that hydrogen and oxygen are actually assimilated, as well as carbon, in the photosynthetic process, the source of the hydrogen and of some of the oxygen being water, taken up from the soil.

[3] For precise methods of gas analysis see: **Bunsen, Robert W. E.,** Gasometrische Methoden. 2te Aufl. Braunschweig, 1877. **Winkler, C. A.,** Lehrbuch der technischen Gasanalyse. 1885. [**Idem,** Handbook of technical gas-analysis containing concise instructions for carrying out gas-analytical methods of proved utility. Translated with a few additions by George Lunge. London, 1885. **Idem,** same title, 2d English ed. from 3d German ed. London, 1902.] For physiological experiments, see especially: **Doyère, M. L.,** Etudes sur la respiration. Ann. chim. et phys. *III*, **28**: 5–50. 1850. **Blackman, F. Frost,** Experimental researches on vegetable assimilation and respiration. I. On a new method for investigating the carbonic acid exchanges of plants. Phil. trans. Roy Soc. London **B186**: 485–502. 1896. [**Idem,** same title. II. On the paths of gaseous exchange between aerial leaves and the atmosphere. *Ibid.* **B186**: 503–562. 1896.] **Palladin, W.,** and **Kostytschew, S.,** in Abderhalden's Handbuch der biochemischen Arbeitsmethoden **3**: 479. Berlin, 1910.

(comparatively pure oxygen[1]) given off, in light, from the cut end of a piece of the water plant Elodea, submerged in water saturated with carbon dioxide, as shown in Fig. 2. If a number of such green water plants are placed under water in sunlight and are covered by an inverted funnel, over the neck of which is inverted a test-tube of water (Fig. 3), the test-tube soon becomes filled with a gas that is nearly pure oxygen.

Schützenberger's reagent (a solution of indigo carmine or nigrosine, decolorized by sodium sulphite) can also be used to demonstrate that oxygen is

FIG. 1.—Leaf in position in a measuring tube, for demonstration of absorption of carbon dioxide and elimination of oxygen during photosynthesis.

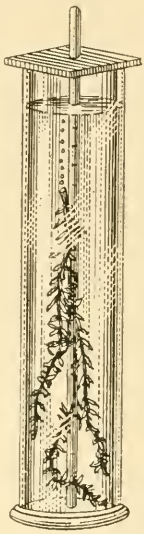


FIG. 2.—Elimination of oxygen bubbles by Elodea in sunlight.

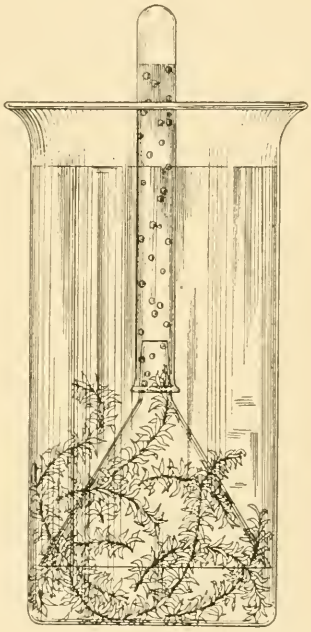


FIG. 3.—Collection of oxygen from water plants in light.

liberated by water plants; this solution is yellow when prepared, but turns blue in the presence of oxygen. If a shoot of Elodea, or other aquatic, is placed in a dilute solution of this reagent and exposed to sunlight, the solution surrounding the leaves becomes blue in a few minutes.[2]

§3. **Chlorophyll.**—Since the decomposition of carbon dioxide is effected exclusively by the green parts of plants, the properties of the green pigment—called

[1] This method was perfected by Kohl. See: **Kohl, F. G.**, Die assimilatorische Energie der blauen und violetten Strahlen des Spektrums. Ber. Deutsch. Bot. Ges. **15**: 111–124. 1897.

[2] **Kny, L.**, Die Abhängigkeit der Chlorophyllfunction von den Chromatophoren und vom Cytoplasma. Ber. Deutsch. Bot. Ges. **15**: 388–403. 1897. [See also: **Kolkwitz, R.**, Pflanzenphysiologie. Jena. 1914. P. 3.]

chlorophyll by Pelletier and Caventou[1]—must be studied. Chlorophyll can be extracted from leaves by alcohol, but the solution thus obtained also contains several other pigments, as well as colorless substances, for the removal of which various methods have been devised.[2] The method of Fremy involves the precipitation of the alcoholic extract with barium hydroxide; the green precipitate is collected upon a filter and treated with alcohol until the yellow pigments, xanthophyll and carotin, are completely removed. The remaining precipitate is then decomposed by potassium hydroxide, according to the method of Timiriazev.[3] The green solution thus obtained is treated with ether, and dilute acetic acid is gradually added, with shaking, to neutralize the potassium hydroxide. As long as the reaction is alkaline the ether remains without color, but as soon as the hydroxide is neutralized the lower layer becomes yellow, since all the green pigment passes into solution in the ether above. The color of the ether solution is emerald green, more intense than that of the alcoholic solution; it is cherry red, however, in reflected light, while the yellow solution shows no fluorescence. Timiriazev was the first to succeed in separating chloroyhyll from yellow pigments, out of the alcoholic chlorophyll extract. This chlorophyll is not the normal pigment, however, for it has been changed by the treatment employed in separating it.

The method of Kraus[4] is based upon the relative solubilities of the pigments in alcohol and benzine. If benzine is gradually added, with shaking, to the green alcoholic extract diluted with water so as to be about an 85-per cent. solution of alcohol, two sharply distinct layers are finally formed, an upper, green layer (benzine) and a lower, golden-yellow one (alcohol and water). By renewed shaking of the former solution, with further additions of alcohol, the green pigment can be practically freed from the yellow coloring matter.

The green pigments[b] form an amorphous mass, readily soluble in alcohol, ether and naphtha. The solution is intensely fluorescent, appearing cherry red by reflected light and green by transmitted light. The chemistry of chlorophyll has been largely worked out by Willstätter and his co-workers. Two closely related pigments are always associated to form chlorophyll, these having been termed *chlorophyll a* and *chlorophyll b*.

[1] [**Pelletier, [Joseph]**, and **Caventou, [J. B.]**, Sur la matière verte des feuilles. Ann. chim. et phys. *II*, 9: 194–196. 1818.]

[2] **Willstatter, Richard**, Chlorophyll und seine wichtigsten Abbauprodukte. Abderhalden's Handb. biochem. Arbeitsmeth. 2: 671–716. Berlin, 1910. **Willstatter, Richard**, and **Hug, Ernst**, Isolierung des Chlorophylls. Liebig's Ann. Chem. u. Pharm. 380: 177–211. 1911.

[3] **Timiriazev, K. A.**, Spectral analysis of chlorophyll. [Russian.] St. Petersburg, 1871.* [**Haas** and **Hill** give methods for obtaining chlorophyll, and present a good discussion. See: **Haas, Paul**, and **Hill, T. G.**, An introduction to the chemistry of plant products. London, 1921.]

[4] **Kraus, Gregor**, Zur Kenntnis der Chlorophyllfarbstoffe und ihrer Verwandten. Stuttgart, 1872.

[b] Some modifications have been made in this discussion of chlorophyll, so that it does not agree entirely with Palladin's presentation. An attempt has been made to bring it more into accord with Willstätter and Stoll's monograph. (**Willstatter, Richard**, and **Stoll, Arthur**, Untersuchungen über Chlorophyll, Methoden und Ergebnisse. Berlin, 1913.) For English résumés of this work, see: **West, Clarence J.**, A review of Willstätter's researches on chlorophyll. Biochem. bull. 3: 229–258. 1914. **Willstatter, R.**, Chlorophyll. Jour. Amer. Chem. Soc. 38: 323–345. 1915.—*Ed.*

Alcoholic solution of chlorohyll *a* is blue-green by transmitted light and blood-red by reflected light; it is said to fluoresce blood-red. Alcoholic solution of chlorophyll *b* is yellow-green by transmitted light and fluoresces brown-red. This phenomenon of fluorescence (seen also in a solution of the red dye eosin, which fluoresces green) appears to be due to an alteration in the wave-length of radiant energy, brought about by a peculiar action on the part of the molecules in the solution. By this action the chlorophyll solution gives off energy of long wave-lengths (red light) when it is illuminated by energy of much shorter wave-lengths (green and blue light).[c]

Of the total green pigment, as obtained from leaves, about 72 per cent. is chlorophyll *a* and the rest chlorophyll *b*. The proportions vary somewhat, but the variation is not over 10 perc ent. Both form crystals. The two chlorophylls[d] have the following formulas, as so far known:

Chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$

It is seen that both contain magnesium, the content of this element being about 5.6 per cent., by weight. Iron is apparently necessary for the formation of chlorophyll in plants, but it is not a part of the pigment. Iron does occur, however, in the molecule of hemoglobin, which is somewhat closely related to chlorophyll, chemically. An explanation[e] of this is to be found in the fact that the actions of these two substances in the cell are directly opposed; for the analytic action of hemoglobin, iron is essential, while magnesium seems to take part in the synthetic processes effected by chlorophyll.[1]

[1] **Willstätter, Richard,** Zur Kenntniss der Zusammensetzung des Chlorophylls. Liebig's Ann. Chem. u. Pharm. **350**: 48–82. 1906. **Willstatter, Richard,** and **Benz, Max,** Ueber krystallisirtes Chlorophyll. *Ibid.* **358**: 267–287. 1908.

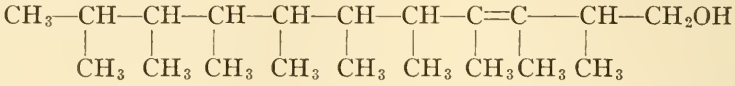
[c] This explanation is not given by Palladin. For a discussion of the various theories regarding the color and fluorescence of plant pigments, see: **Horowitz, B.,** Plant pigments. Biochem. bull. **4**: 161–172. 1915.—*Ed.*

[d] Stokes had long ago suspected that chlorophyll is a mixture of two green pigments. In this connection see: **Stokes, G. G.,** On the supposed identity of biliverdin with chlorophyll, with remarks on the constitution of chlorophyll. Proc. Roy. Soc. London **13**: 144–145. 1864. **Sorby, H. C.,** On comparative vegetable chromatology. *Ibid.* **21**: 442–483. 1873.

On an interesting method for separating the yellow and green pigments by absorption in paper or in a column of calcium carbonate, see: **Tswett, M.,** Physikalisch-chemische Studien über das Chlorophyll. Die Adsorptionen. Ber. Deutsch. Bot. Ges. **24**: 316–323. 1906. **Idem,** Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. *Ibid.* **24**: 384–393. 1906. **Idem,** Ueber die nächsten Säurederivate der Chlorophylline. Ber. Deutsch. Chem. Ges. **41***I*: 1352–1354. 1908.—*Ed.*

[e] It is difficult to understand this as an *explanation.* It must not be understood that hemoglobin and chlorophyll are really very much alike; they differ very markedly, but give some of the same decomposition products. It is true that both are related to the interchange, between the organism and its surroundings, of carbon dioxide and oxygen, but the actions of the two substances do not appear to be similar in detail. The author refers here to the fact that they have similar component atomic groups, which may suggest that, in the phylogeny of animals and plants, both groups of organisms may have developed from a common ancestral form having a substance with the characters that are common to hemoglobin and chlorophyll. This is as far as such a theory may go at present. But see below, page 11, *et seq.*—*Ed.*

Almost a third of the chlorophyll molecule is composed of phytyl, the radical of phytol,[1] an unsaturated mono-hydric primary alcohol of the aliphatic series, having the composition $C_{20}H_{40}O$ and the probable structure shown by the following diagram:



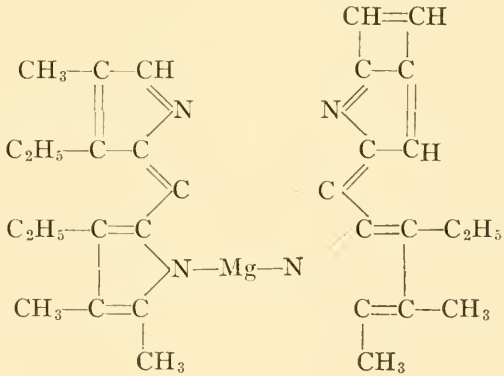
Phytol is readily oxidized in the presence of air. Willstätter suggests that it may be obtained from isoprene in the following way:

$$4C_5H_8 \text{ (isoprene)} + H_2O + 3H_2 = C_{20}H_{40}O \text{ (phytol)}.$$

Carotin appears also to be related to isoprene. The phytyl of chlorophyll may be replaced by the ethyl group if the leaves are treated with ethyl alcohol. This replacement is effected by an enzyme known as chlorophyllase.[2]

Another alcohol radical is present in both the chlorophylls, namely, methyl (CH_3). They thus appear to be esters of a complex, dicarboxylic acid, one of the two carboxyls (COOH) being joined to phytyl and the other to methyl.

Regarding the complex acids that form the basis of the chlorophylls, there still remain some uncertainties, but it appears to be related to a tricarboxylic acid that may be represented by the formula $(C_{31}H_{29}N_4Mg)\ (COOH)_3$, but one of the carboxyls is inactive, so that a dicarboxylic acid results. A general idea of the manner in which the magnesium atom is probably related to the other components of the molecule may be obtained from the following structural formula for *etiophyllin*, to which this fundamental acid is apparently closely related.



When the phytyl group of chlorophyll *a* is replaced by the ethyl group (C_2H_5), a substance is obtained ($C_{37}H_{38}O_6N_4Mg$) which Willstätter called *ethyl chlorophyllide*. This forms beautiful crystals, which were earlier mistaken for pure chlorophyll. Chlorophyll *b* reacts in a similar way. According to the

[1] **Willstätter, Richard,** and **Hocheder, Ferdinand,** Ueber die Einwirkung von Säuren und Alkalien auf Chlorophyll. Liebig's Ann. Chem. u. Pharm. **354**: 205–258. 1907. **Willstätter, Richard, Mayer, Erwin W.,** and **Huni, Ernst,** Ueber Phytol. I. *Ibid.* **378**: 73–152. 1911.

[2] **Willstatter, Richard,** and **Stoll, Arthur,** Ueber Chlorophyllase. Liebig's Ann. Chem. u. Pharm. **378**: 18–72. 1911.

method of Monteverde,[1] these crystals may be obtained by treatment of triturated leaves with 95 per cent. ethyl alcohol; after an hour the extract is filtered and the alcohol is removed by evaporation, either in air or in hydrogen. The crystals are separated from impurities and from the yellow pigments by means of distilled water and benzine. In the pure condition they form a dark green, almost black powder, with a bluish metallic luster. Their alcoholic solution is green, with a beautiful red fluorescence. Although the solution is unstable in light, the crystals can endure intense light for a long time without change. The following plants serve especially well as sources of ethyl chlorophyllide in the crystalline condition: *Dianthus barbatus*, *Lathyrus odoratus*, *Galeopsis versicolor*, *G. tetrahit*, *Acacia lophantha*, and *Dahlia variabilis*. Amorphous chlorophyll may be obtained from many other plants. Willstätter and Benz[2] obtained over 2 g. of ethyl chlorophyllide from 1 kg. of dry leaves.

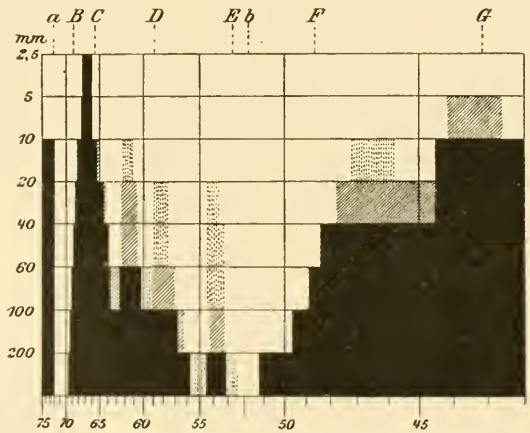


FIG. 4.—Absorption spectrum of ethyl chlorophyllide, 0.1 g. in 5 l. of alcohol. (*After Willstätter.*) The thickness of the layer employed is shown (in millimeters) at the left, the conventional letters of the Fraunhofer lines are at the top, and the wave-lengths (in 10 $\mu\mu$) are indicated below.

The absorption spectrum of chlorophyll deserves special attention. Light of certain ranges of wave-length is more or less completely absorbed by the solution, so that dark bands appear in the spectrum. The absorption spectrum of every colored solution changes with its concentration. On this account the spectrum of chlorophyll solution must be determined either throughout a range of concentrations or by using layers of various thicknesses. Six absorption bands are found in the spectrum (Fig. 4) of ethyl chlorophyllide; arranged according to their intensities, they form the series: I, VI, V, II, III, IV. The first band, lying between the Fraunhofer lines B and C, is the most distinct; it appears in solutions of weaker concentration than are necessary to make the others evident. The absorption bands become broader with increasing con-

[1] **Monteverde, N. A.,** Ueber das Protochlorophyll. Acta Horti Petropolitani **13**: 199–217. 1894. Borodin had obtained crystals from chlorophyll before they were described by Monteverde. See: **Borodin, J.,** Ueber Chlorophyllkrystalle. Bot. Zeitg. **40**: 608–610, 622–626. 1882.

[2] **Willstätter** and **Benz,** 1908. [See note 1, p. 7.]

centration and finally merge into one another, so that only the red rays, between *A* and *B*, and a part of the green can pass through a concentrated solution or a thick layer; finally, with still further increase in concentration or thickness

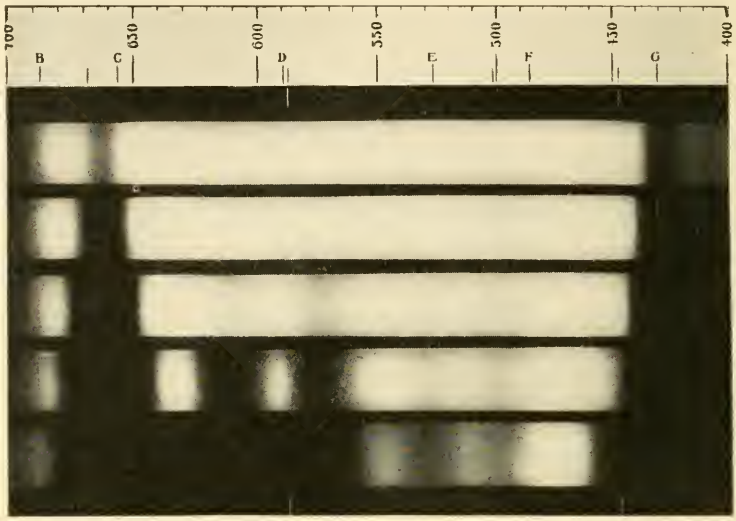


FIG. 5.—Absorption spectra of five different concentrations of chlorophyll *a*. (*After Willstätter and Stoll.*)

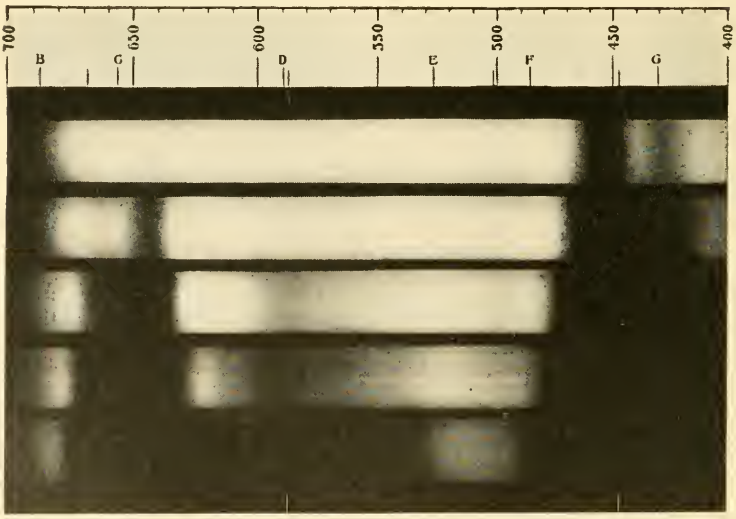


FIG. 6.—Absorption spectra of five different concentrations of chlorophyll *b*. (*After Willstätter and Stoll.*)

of layer, the green rays are also completely absorbed and only the rays between *A* and *B* are transmitted. All objects appear red when seen through a very concentrated solution or a very thick layer.

The absorption spectra of chlorophyll *a* and chlorophyll *b*, in acetone, are shown in Figs. 5 and 6, reproduced photographically, these being taken from Willstätter and Stoll's monograph (Tafel VIII). Five different concentrations are employed, the strongest being represented by the lowest spectrum in each case. The Fraunhofer lines and wave-lengths (in $\mu\mu$) are shown above.[f]

The spectrum of living leaves shows the same absorption bands as does the spectrum of an alcoholic solution of chlorophyll (ethyl chlorophyllide); in the former case the bands are merely displaced a little toward the infra-red end of the spectrum.[g]

The researches of Schunck and Marchlewski[1] have contributed much to an understanding of the chemical character of chlorophyll. The action of hydrochloric acid upon an alcoholic chlorophyll solution produces first *chlorophyllan*, then *phylloxanthin*, and finally *phyllocyanin*. The interesting substance *phylloporphyrin*[2] ($C_{16}H_{18}N_2O$, or $C_{32}H_{36}N_4O_2$)[3] is obtained by treating phyllocyanin with strong alkalies. Phylloporphyrin crystallizes in beautiful, dark red-violet

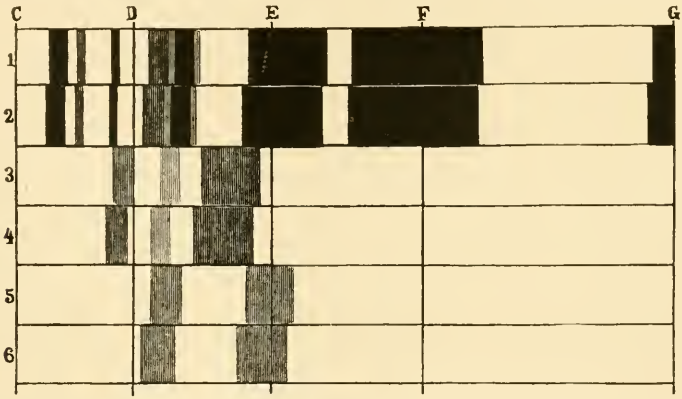


FIG. 7.—Absorption spectra of phylloporphyrin (1, 3, 5) and of hematoporphyrin (2, 4, 6); 1 and 2 in ether; 3 and 4 in hydrochloric acid; 5 and 6 in zinc chloride solution. (*After Schunck and Marchlewski.*)

crystals, is slightly soluble in alcohol and ether, and more readily soluble in chloroform. The absorption spectrum of its ethereal solution (Fig. 7) exhibits seven absorption bands, the first of which lies to the right of the red region of the spectrum, between *C* and *D*, and is very distinct.

Phylloporphyrin is of great interest because of its close relationship to

[1] **Schunk, E.,** and **Marchlewski, L.,** Zur Chemie des Chlorophylls. Liebig's Ann. Chem. u. Pharm. **278**: 329–345. 1894.

[2] **Schunck, E.** and **Marchlewski; L.,** Zur Chemie des Chlorophylls. (Zweite Abhandlung.) Liebig's Ann. Chem. u. Pharm. **284**: 81–107. 1895.

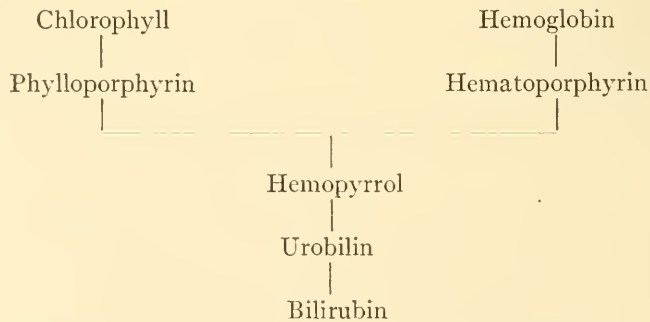
[3] **Willstätter, Richard,** and **Fritzsche, Hermann,** Ueber den Abbau von Chlorophyll durch Alkalien. Liebig's Ann. Chem. u. Pharm. **371**: 33–124. 1909.

[f] These two figures are added by the editor.—*Ed.*

[g] It seems highly probable that the chlorophyll of living leaves exists in colloidal solution. (**Herlitzka, A.,** Neben den Zustand des Chlorophylls in der Pflanze und über kolloidales Chlorophyll. Biochem. Zeitsch. **38**: 321–330. 1912. **Iwanowski, [D.],** Ueber das Verhalten des lebenden Chlorophylls zum Lichte. Ber. Deutsch. Bot. ges. **31**: 600–612. 1913).—*Ed.*

hematoporphyrin, which was obtained by Nentskii and Sieber from hemoglobin of animal blood. Hematoporphyrin has the composition $C_{16}H_{18}N_2O_3$, the difference between it and phylloporphyrin, as represented by these formulas, consisting in the lower oxygen content of the latter.[1] The method used in the isolation of hematoporphyrin is also analogous to that employed for phylloporphyrin. The spectra of these two substances, in various solvents,[2] are almost identical, except that the absorption bands of hematoporphyrin sometimes appear slightly displaced toward the red (Fig. 7).

Both hematoporphyrin and phylloporphyrin, when heated in a test-tube, form a vapor which gives a red color to pine sawdust moistened with hydrochloric acid, a characteristic indication of the presence of the pyrrol ring (C_4H_5N): the characteristic odor of pyrrol may also be plainly recognized in this vapor.[3] It thus appears that chlorophyll (acting synthetically) and hemoglobin (acting analytically) are closely related, in that the pyrrol ring is common to both. It is of great interest also to note that the bile pigment *bilirubin* has the same percentage formula as hematoporphyrin ($C_{16}H_{18}N_2O_3$). Furthermore, Nentskii and Zaliesskii[4] succeeded in obtaining *mesoporphyrin* from *hemin*, the latter substance being formed by the action of acids upon hemoglobin. Mesoporphyrin has the composition $C_{16}H_{18}N_2O_2$, and stands between hematoporphyrin and phylloporphyrin in oxygen content. By a further decomposition of hemin these authors obtained *hemopyrrol* ($C_{13}H_8N$), a volatile oil that turns red in air and changes into *urobilin*, which is also obtained from bilirubin. When Nentskii and Marchlewski[5] succeeded in obtaining hemopyrrol and urobilin from phylloporphyrin, the relationship between chlorophyll and hemoglobin was conclusively established. The atomic group common to both, as in the case of the bile pigments, occurs in hemopyrrol. The following diagram represents the relationship existing between these three groups of substances.



[1] For the difference in structure between the two compounds see: **Willstätter, Richard,** and **Asahina, Yasuhiko,** Oxydation der Chlorophyllderivate. Liebig's Ann. Chem. u. Pharm. **373**: 227–238. 1910.

[2] **Schunck, E.,** and **Marchlewski, L.,** Zur Chemie des Chlorophylls. (Vierte Abhandlung.) Liebig's Ann. Chem. u. Pharm. **290**: 306–313. 1896.

[3] **Schunck, E.,** and **Marchlewski, L.,** Zur Chemie des Chlorophylls. (Dritte Abhandlung.) Liebig's Ann. Chem. u. Pharm. **288**: 209–218. 1895.

[4] **Nencki, M.,** and **Zaleski, J.** Ueber die Reductionsproducte des Hämins durch Jodwasserstoff and Phosphoniumjodid und über die Constitution des Hämins and seiner Derivate. Ber. Deutsch. Chem. Ges. 34^{I}: 997–1010. 1901.

[5] **Nencki, M.,** and **Marchlewski, L.,** Zur Chemie des Chlorophylls. Abbau des Pyhllocyanins zum Hämopyrrol. Ber. Deutsch. Chem. Ges. 34^{II}; 1687–1693. 1901.

Results of this kind are exceedingly important in biochemistry, since they seem to illuminate the most remote period in the evolutionary development of organisms, and point to a common origin of the plant and animal worlds. Darwin's theory of the origin of species is based upon the conception of variability in structure, influenced by environmental conditions in the struggle for existence. But the differences between organisms lie, not only in the form and structure of the various organs, but also in the chemical properties of the substances constituting the living cells. The character of the metabolic processes is dependent upon the nature of the intracellular substances, and these processes, in their turn, determine the configuration of the cells and their differentiation into organs. In other words, the form of the cell-complexes composing the different organs is determined by metabolism as this has been developed by the various organs in the struggle for existence, relative to various environmental conditions. With a change of conditions, their chemical constitution and their metabolism are modified, which explains why they frequently change their form also. Thus, to obtain a fundamental conception of the evolution of the organic world, not only the structure but also the chemical composition of the cells and the products of their metabolism must be considered. From this viewpoint the work of Schunck and Marchlewski, whereby the leaf and blood pigments are shown to be related chemically, though widely different as to function, is of great scientific interest.[1]

According to Nentskii,[2] chlorophyll and hemoglobin arise from chromogens that are protein decomposition products. A substance called *tryptophan* is formed in protein decomposition by pancreatic juice; tryptophan is colored red by bromine and is related, in its percentage composition, to hematoporphyrin and the melanins.

The decomposition products of chlorophyll can be separated, according to Willstätter,[3] into two groups. Those obtained by the action of acids contain no magnesium; the action of alkalies, on the other hand, results in such derivatives as *glaucophyllin*, *rhodophyllin*, *pyrrophyllin*, and *phyllophyllin*, all of which contain magnesium. If acids are allowed to act upon these latter substances, new compounds without magnesium arise, which are related to hematoporphyrin; in this way phylloporphyrin is obtained from phyllophyllin. The action of acids upon chlorophyll itself gives *phæophytin*, in which the phytyl can be replaced by the ethyl group, giving *ethyl phæophorbide;* chlorophyllin modified by the action of acid is designated as phæophorbide, and phæophytin may thus be termed phytyl-phæophorbide.

[1] **Nencki, M.**, Sur les rapports biologiques entre la matière colorante des feuilles et celle du sang. Arch. sci. biol. St.-Pétersbourg **5**: 254–260. 1897.

[2] **Nencki, M.**, Ueber die biologischen Beziehungen des Blatt- und des Blutfarbstoffes. Ber. Deutsch. Chem. Ges. **29**^{III}: 2877–2883. 1896.

[3] **Willstatter, Richard,** and **Pfannenstiel, Adolf,** Ueber Rhodophyllin. Liebig's Ann. Chem. u. Pharm. **358**: 205–265. 1908. **Willstatter** and **Fritzsche,** 1909. [See note 3, p. 11.] **Willstatter** and **Hocheder,** 1907. [See note 1, p. 8.] **Willstatter, Richard,** and **Stoll, Arthur,** Spaltung und Bildung von Chlorophyll. Liebig's Ann. Chem. u. Pharm. **380**: 148–154. 1911. **Willstatter, Richard,** and **Isler, Max.,** Vergleichende Untersuchung des Chlorophylls verschiedener Pflanzen. III. *Ibid.* **380**: 154–176. 1911. [The whole series of studies is summarized by **Willstatter** and **Stoll,** 1913. (See note *b*, p. 6.)

Among the other transformation products of chlorophyll, *protophyllin* deserves attention; Timiriazev[1] obtained this by the action of nascent hydrogen. It is yellow or red in solution, according to the concentration. It is very easily oxidized, going over into chlorophyll; for this reason it must be preserved under carbon dioxide or hydrogen in sealed tubes. It is stable in hydrogen, in light as well as in darkness, but in carbon dioxide it is stable only in darkness; in light, with carbon dioxide, it becomes green and is transformed into chlorophyll. It must be supposed that carbon dioxide is decomposed in this case and that oxygen is liberated, at the expense of which the transformation and greening of the protophyllin occurs. Absorption bands in the orange and green regions of the spectrum, corresponding to bands II and IV of chlorophyll, are characteristic of protophyllin.

It appears from many investigations that the formation of chlorophyll in plants is a very complicated process. Until the publication of the work of Liro[2] most authors failed to distinguish between the beginning of chlorophyll *formation* and the visible *accumulation* of this pigment in plants as they become green. This distinction is quite necessary.

We shall first turn our attention to the conditions requisite for the formation of chlorophyll. Light may be mentioned as the first of these. Leaves of angiosperms grown in darkness are always yellow, but such *etiolated* plants soon turn green when exposed to light. Seedlings of some conifers,[3] young fern fronds and some one-celled algæ[4] are exceptions, for they become green in darkness; still, according to Liubimenko, conifer seedlings form much less chlorophyll in darkness than in light. Very weak light is sufficient for chlorophyll formation, and light of medium intensity is most favorable. Famintsyn[5] exposed a part of an etiolated plant to direct sunlight, while the intensity of the light falling upon the remaining portion was reduced by interposing sheets of paper; greening always occurred first in the reduced light. According to Wiesner this phenomenon is to be explained by supposing that decomposition and formation of chlorophyll occur simultaneously. In light of low or medium intensity the decomposition process is nearly absent, while in strong light active formation is accompanied by rapid breaking down of chlorophyll, which results in less pronounced greening than occurs in diffuse light.

Various parts of the spectrum have different effects upon the formation of chlorophyll, a matter which was carefully investigated by Wiesner.[6] He

[1] **Timiriazeff, C.**, La chlorophylle et la réduction de l'acide carbonique par les végétaux. Compt. rend. Paris **102**: 686–689. 1886. **Idem**, La protophylline dans les plantes etiolées. *Ibid.* **109**: 414–416. 1889. **Idem**, La protophylline naturelle et la protophylline artificielle. *Ibid.* **120**: 467–470. 1895.

[2] **Liro, J. Ivar**, Ueber die photochemische Chlorophyllbildung bei den Phanerogamen. Ann. Acad. Sci. Fennicæ (Helsinki) **A1**: 1–147. 1909.

[3] **Lubimenko, W.**, Influence de la lumière sur le développement des fruits et des graines chez les végétaux superieurs. Rev. gén. bot. **22**: 145–175. 1910.

[4] **Artari, A.**, Ueber die Entwicklung der grünen Algen unter Ausschluss der Bedingungen der Kohlensäure-Assimilation. Bull. Soc. Imp. Nat. Moscou **13**: 39–47. 1900. **Idem**, Zur Ernährungs-physiologie der grünen Algen. Ber. Deutsch. Bot. Ges. **19**: 7–9 1901.

[5] **Famintzin, A.**, Die Wirkung des Lichts auf das Ergünen der Pflanzen ("aus dem Bulletin **10**: 548–552.") Mélanges biol. Acad. Imp. Sci. St.-Pétersbourg **6**: 94–100. 1866.

[6] **Wiesner, Julius**, Untersuchungen über die Beziehungen des Lichtes zum Chlorophyll. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **69**^{I}: 327–385. 1874. **Idem**, Die Entstehung des Chlorophylls in der Pflanze. Wien, 1877.

employed double-walled bell-jars with colored liquids, as light screens for isolating certain regions of the spectrum (Fig. 8). Solutions of potassium dichromate and of ammoniacal copper oxide [copper sulphate solution to which an excess of ammonia water is added] were most frequently used; the first, in medium concentration, permits the passage of the rays of the less refrangible half of the spectrum (red, orange, yellow and a part of the green), while the second transmits the remainder of the visible rays (the rest of the green and all of the blue and violet). Thus, by the use of these liquids, the spectrum is separated into two parts. [Of course the intensity of the light transmitted is considerably decreased.]

In weak light plants become green sooner under the yellow solution, but in strong light more quickly under the blue. This may be explained by supposing that in weak light the *formation* of chlorophyll occurs almost exclusively, under the influence of the less refrangible rays, which are most favorable, while in strong light, besides chlorophyll formation, an active *decomposition* also takes place. Experiments upon the decomposition of alcoholic solutions of chlorophyll under colored bell-jars have shown that this process is especially pronounced in the less refrangible half of the spectrum; greening in plants is thus seen to be weaker in strong yellow-red light because a very rapid destruction here accompanies the formation of chlorophyll. But another explanation is also possible: strong light may not act directly upon chlorophyll that has already been formed but may, somehow, have a harmful effect upon some process antecedent to chlorophyll formation; this might explain why less chlorophyll accumulates in strong light.

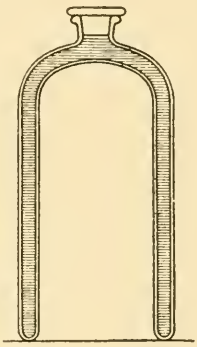


Fig. 8.—Double-walled bell-jar with colored solution filling the space between the walls.

Plants do not become green under the non-luminous heat rays. In order to separate this portion of the spectrum, Tyndall's solution is used, iodine in carbon bisulphide; in low concentrations the rays between Fraunhofer lines *A* and *B* are transmitted, but these, produce no green color. In ultra-violet light greening is very slight.

The production of chlorophyll is dependent upon temperature. Medium temperatures are most favorable, and no greening occurs at very low or at very high temperatures. Wiesner obtained the following results from experiments with etiolated barley seedlings.

Temperature *Deg.C.*	Time Required for Greening *Hours*
2– 4	(No greening)
4– 5	7.25
10	3.50
18–19	1.67
30	1.58
37–38	4.00
40	(No greening)

The autumn coloration of leaves is dependent upon light and upon the temperature of the air; chlorophyll is decomposed by sunlight in autumn, while its re-formation is hindered by the low temperatures then prevailing. According to Batalin,[1] the conifer *Chamæcyparis obtusa* is especially interesting in this connection. Branches in sunshine have a golden-yellow color in the cold season, while shaded ones remain green;[h] at the margin between the shaded and unshaded regions the different colors may often be seen in neighboring cells.

The products of chlorophyll decomposition do not remain in the leaf but diffuse away.[2] This is shown by the following experiment: if an incision is made in a leaf in the autumn, while it is still green, so that the chlorophyll decomposition-products are prevented from diffusing away, the part of the leaf above the cut remains green while the other parts turn yellow (Fig. 9).

The presence of iron is a third condition necessary for the formation of chlorophyll.[3] Without iron, plants remain bright yellow, thus suffering from *chlorosis*.

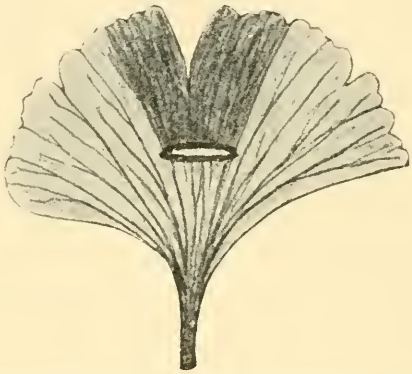


FIG. 9.—Gingko leaf in which autumnal coloration has been prevented in the upper part, by an incision. (*After Stahl.*)

The presence of oxygen is an additional condition necessary for greening. Etiolated leaves in an oxygen-free chamber remain yellow, even in light. This is also true when the amount of oxygen is small; greening demands an excess of this gas.

Ville[4] was able to show that the absence of necessary mineral salts in the soil results in the diminution of the chlorophyll and carotin contents of leaves.

[1] Batalin, A., Ueber die Zerstörung des Chlorophylls in lebenden Organen. Bot. Zeitg. 32: 433–439. 1874.

[2] Stahl, Ernst, Zur Biologie des Chlorophylls; Laubfarbe und Himmelslicht, Vergilbung und Etiolement. Jena, 1909.

[3] Gris, Eusèbe, Nouvelles expériences sur l'action des composés ferrugineux solubles, appliqués à la végétation, et spécialement au traitement de la chlorose et de la débilité des plantes. Compt. rend. Paris 19: 1118–1119. 1844. Molisch, Hans, Die pflanze in ihren Beziehungen zum Eisen. Eine physiologische Studie. Jena, 1892.

[4] Ville, Georges, Recherches sur les relations qui existent entre la couleur des plantes et la richesse des terres en agents de fertilité. Compt. rend. Paris 109: 397–400. 1889.

[h] This may also be seen in the arbor vitæ (*Thuja occidentalis*) of the northeastern United States in very cold, bright winter weather.—*Ed.*

Lesage and Schimper[1] found that an excess of mineral substances reduces the chlorophyll content, an effect that may be observed not only in halophytes, growing normally upon soils rich in salts, but also in other plants when watered with strong salt solutions.

Finally, Palladin[2] pointed out that carbohydrates are essential to the formation of chlorophyll. As will be seen farther on, plants fall into two groups according to the carbohydrate content of their etiolated leaves; in one group (for example, wheat), such leaves contain much soluble carbohydrate material, while in etiolated leaves of the other group (such as bean and lupine) carbohydrates are almost entirely absent. If etiolated leaves of these plants are removed and floated upon water in light, those of barley become green, while almost all the bean leaves and all those of lupine remain yellow. In the latter are floated, not upon water but upon a saccharose or glucose solution, then they also all become green. The greening of entire, completely etiolated bean plants in light is explained in this way, that carbohydrates migrate into the leaves from the stems. Besides saccharose and glucose, such substances as raffinose, fructose, maltose, glycerine, and some others, also produce greening[3] under these conditions. The concentration of these substances is important in this connection.[4] Greening occurs quickly with a saccharose solution of low or medium concentration. If the concentration is previously increased to 35 per cent., in darkness, the leaves remain yellow for several days when subsequently brought into the light, but greening occurs quickly in these leaves if they are transferred from the strong solution to one having a concentration of from 5 to 10 per cent.

Single-celled algæ are particularly well adapted to the study of the importance of various substances in the formation of chlorophyll. Cultures in light exhibit a considerable range of color (from yellow-green to intense, dark green) according to the composition of the nutrient solution used.[5]

Thus greening, or the accumulation of chlorophyll, is a physiological process that proceeds only in living cells and under conditions favorable to life. The substance from which chlorophyll arises has not yet been isolated, but the existence of such a substance may be inferred from various observations. According to Monteverde and Liubimenko,[6] a pigment called *chlorophyllogen* is formed, independently of light, in the chromatophores of all green plants. It is said to arise from a colorless chromogen, *leucophyll*,[7] of which little more is

[1] **Schimper, A. F. W.,** Die Indo-Malayische Strandflora. Jena, 1891. P. 9.

[2] **Palladin, W.,** Ergrünen und Wachsthum der etiolirten Blätter. Ber. Deutsch. Bot. Ges. 9: 229–232. 1891.

[3] **Palladin, W.,** Recherches sur la formation de la chlorophylle dans les plantes. Rev. gén. Bot. 9: 385–394. 1897.

[4] **Palladin, W.,** Einfluss der Concentration der Lösungen auf die Chlorophyllbildung in etiolirten Blättern. Ber. Deutsch. Bot. Ges. 20: 224–228. 1902.

[5] **Artari, Alexander,** Ueber die Bildung des Chlorophylls durch grüne Algen. Ber. Deutsch. Bot. Ges. 20: 201–207. 1902. **Matruchot, L.,** and **Molliard, M.,** Variations de structure d'une algue verte sous l'influence du milieu nutritif. Rev. gén. bot. 40: 114–130, 254–268. 1902.

[6] **Monteverde, N. A.,** and **Lubimenko, V. N.,** Recherches sur la formation de la chlorophylle chez les plantes. [Text in Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI.* 5: 73–100. 1911.

[7] **Sachs, J.,** Ueber das Vorhandensein eines farblosen Chlorophyll-Chromogens in Pflanzentheilen, welche fähig sind grün zu werden. Lotos 9: 6–14. 1859. **Idem,** same title. Chem. Centralbl., n. F. 4: 145–153. 1859.

known. Chlorophyllogen is a very unstable substance, and its absorption spectrum shows a great similarity, in the red region, to that of chlorophyll. Attempts to isolate it result in an artificial transformation-product, the *protochlorophyll* of Monteverde.[1] Like chlorophyll, protochlorophyll is a deep green pigment, which is fluorescent, appearing red by reflected light. The spectrum shows four absorption bands. The absorption spectra of alcoholic solutions of protochlorophyll on the one hand, and of alcoholic chlorophyll on the other, are different in that the absorption band between *B* and *C* in the second is absent in the first, and the one between *C* and *D* in the first appears slightly displaced toward the left in the second; the other bands practically agree. Although protochlorophyll is a transformation-product, it is still of interest, in so far as its existence indicates the presence of a mother-substance for chlorophyll; protochlorophyll itself cannot change into chlorophyll. Protochlorophyll arises independently of light, from chlorophyllogen. As to its presence in living cells, it is normally found in large quantities in the inner seed-coats of the Cucurbitaceæ, especially in Luffa.

A rapid transformation of chlorophyllogen into chlorophyll occurs in living plant cells under the influence of light. This process can also be observed in plants that have been killed. According to Liro, if etiolated leaves are carefully killed so that at least some of the chlorophyllogen remains, and if they are then exposed to light, some formation of chlorophyll can still be observed. For the transformation of chlorophyllogen into chlorophyll, Liro and Isachenko[2] have shown that neither oxygen, favorable temperature conditions, nor even the presence of carbohydrates are necessary, but since greening is possible only with these conditions they are evidently necessary for the formation of chlorophyllogen, or of the chromogen that gives rise to it. Chlorophyll may be formed from chlorophyllogen in the absence of light, as is exemplified by plants that turn green in darkness; in such cases the influence of chemical agents must replace the action of light.[3]

Such are the chief results of the researches thus far carried out upon chlorophyll and its formation. As to the rôle it plays in the chemical decomposition of carbonic acid and the formation of the first products of photosynthesis almost nothing is known. Schryver[4] suggests that the formaldehyde arising in the decomposition of carbon dioxide and water enters into combination with the chlorophyll.

[1] **Monteverde,** 1894. [See Note, 1, p. 9.] **Monteverde, N. A.,** Der Einfluss des Lichts auf die Geschwindingkeit der Chlorophyllbildung in Blättern etiolirter Pflanzen. Trav. Soc. Imp. Nat. St.-Pétersbourg **27**[1]: 131–142 [Russian], 143–145 [German abstract]. 1896. **Idem,** Das Protochlorophyll und Chlorophyll. [Title and abstract in German, article in Russian.] Bull. Jard. Imp. Bot. St.-Pétersbourg **2**: 179–182. [Abstract, p. 181–182.] 1902. **Idem,** Ueber das Absorptionsspectrum des Protochlorophylls. I. [Title and abstract in German, article in Russian.] *Ibid.* **7**: 37–42 [Abstract, p. 42], 47–58. [Abstract, p. 55–58]. 1907.

[2] **Issatchenko, B.,** Sur les conditions de la formation de la chlorophylle. [Title and abstract in French, article in Russian.] Bull. Jard. Imp. Bot. St.-Pétersbourg **6**: 20–28 [Abstract, p. 27–28]. 1906. **Idem,** same title. *Ibid.* **7**: 59–64 [Abstract, p. 64]. 1907. **Idem,** same title. *Ibid.* **9**: 106–120 [Abstract, p. 119–120]. 1909.

[3] **Monteverde** and **Liubimenko,** 1911. [See note 6, p. 17.]

[4] **Schryver, S. B.,** Photochemical formation of formaldehyde in green plants. Chem. news **101**: 64. 1910.

As to the physics of the action of chlorophyll, it behaves as a sensitizer[1] and renders the energy of the absorbed light effective in the decomposition of carbon dioxide. In an analogous manner the red light rays between lines *B* and *C* of the spectrum rapidly decompose silver salts in the presence of chlorophyll, although these salts are otherwise decomposed only by blue and violet rays.

§4. Pigments Accompanying Chlorophyll.—Among the other pigments accompanying chlorophyll, special attention should be given to *carotin*.[2] Borodin[3] was able to show that carotin (called erythrophyll by him) regularly appeared in alcoholic leaf extract when he allowed this to form crystals under the microscope.

The chemical nature of carotin, and also some of the conditions of its formation in leaves, were first made clear by the investigations of Arnaud[4] and of Willstätter and Mieg.[5] This pigment forms flat, rhombic crystals, which, with one-sided illumination, appear blue-green on the illuminated side and orange-red on the other. It is readily soluble in ether, chloroform and carbon bisulphide, less so in benzine, slightly soluble in hot alcohol, almost insoluble in cold alcohol and insoluble in water. A carbon bisulphide solution of carotin is blood-red; dissolved in concentrated sulphuric acid, carotin is bluish-violet. It is a hydrocarbon, with the formula $C_{40}H_{56}$, which is easily oxidized. It may be transformed into cholesterin. The carotin content of leaves varies with the season of the year. A series of experiments continued throughout the summer upon the leaves of stinging nettle and horse-chestnut showed that the carotin content is greatest during the flowering season, for both plants. The formation of carotin is also dependent upon light; green leaves of vetch contained 178.8 mg. of carotin, as compared to 34.0 mg. in the same quantity of etiolated leaves.

It was shown by the work of Kohl[6] that carotin is widely distributed. It is not limited to the green parts of plants but occurs also in flowers, fruits, seeds and subterranean organs, and also in fungi. It may be extracted in large quantities from carrots.

The function of carotin is not yet clear, but its tendency to unite with oxygen appears, at any rate, to be significant in connection with the photosynthetic process, where reduction of compounds containing oxygen is known to occur.

[1] **Tappeiner, H. von,** Die photodynamische Erscheinung (Sensibilisierung durch fluoreszierende Stoffe). Ergeb. Physiol. **8**: 698–741. 1909.

[2] **Escher, Heinr. H.,** Zur Kenntnis des Carotins und des Lycopins. Zürich, 1909. 104 p. (Zürich Polytechn. Dissert. 1909–10.) [For a general discussion of the yellow pigments, see **Haas** and **Hill**, 1921. (See note 3, p. 6.)]

[3] **Borodin, J.,** Ueber krystallinische Nebenpigmente des Chlorophylls. Bull. Acad. Imp. Sci. St.-Pétersbourg **28**: 328–350. 1883.

[4] **Arnaud, A.,** Recherches sur les matières, colorantes des feuilles; identité de la matière rouge orangé avec la carotine, $C_{18}H_{24}O$. Compt. rend. Paris **100**: 751–753. 1885. **Idem,** Recherches sur la composition de la carotine, sa fonction chimique et sa formule. *Ibid.* **102**: 1119–1122. 1886. **Idem,** Sur la présence de la cholestérine dans la carotte; recherches sur ce principe immédiat. *Ibid.* **102**: 1319–1322. 1886. **Idem,** Recherches sur la carotine; son rôle physiologique probable dans la feuille. *Ibid.* **109**: 911–914. 1889.

[5] **Willstätter, Richard,** and **Mieg, Walter,** Ueber die gelben Begleiter des Chlorophylls. Liebig's Ann. Chem. u. Pharm. **355**: 1–28. 1907.

[6] **Kohl, Friedrich Georg,** Untersuchungen über das Karotin und seine physiologische Bedeutung in der Pflanze. Leipzig, 1902.

The absorption spectrum of carotin has two dark bands in the green-blue half of the spectrum (Fig. 10).

A second yellow pigment accompanying chlorophyll is *xanthophyll*, an oxidation product of carotin, with the formula $C_{40}H_{56}O_2$.[i]

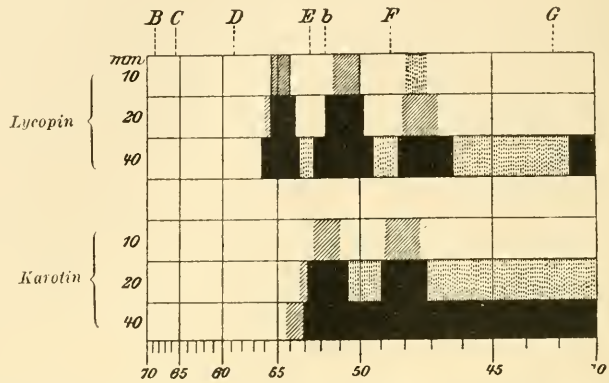


FIG. 10.—Absorption spectra of carotin and lycopin. (*After Escher.*) The Fraunhofer lines are indicated by the letters above and the wave-lengths (in 10 $\mu\mu$) are shown below; the thickness of layer employed is given (in mm.) at the left.

Lycopin[1] is closely related to carotin and has the same percentage formula ($C_{40}H_{56}$); it is found in the fruit of the tomato (*Solanum lycopersicum*). Three dark bands occur in the right half of its absorption spectrum (Fig. 10).

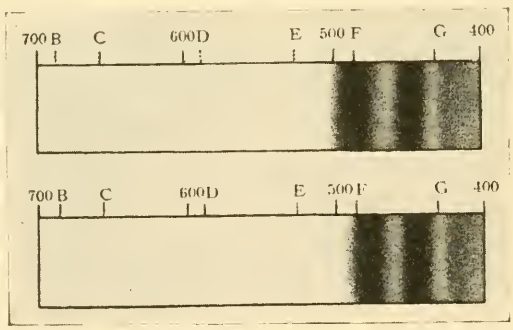


FIG. 11.—Absorption spectra of carotin (above) and xanthophyll (below). (*After Willstätter and Stoll.*) The Fraunhofer lines and the wave-lengths (in $\mu\mu$) are shown on the upper line of each diagram.

Red algæ contain *phycoerythrin*, a protein-like substance, which is readily soluble in water but insoluble in alcohol, ether, and carbon bisulphide. The

[1] **Montanari, Carlo,** Materia colorante rossa del pomodoro. Le Stazioni Sperimentali Agrarie Italiane 37: 909–919. 1904. [**Willstatter, Richard,** and **Escher, Heinr. H.,** Ueber den Farbstoff der Tomate. Zeitsch. physiol. Chem. 64: 47–61. 1910.]

[i] The absorption spectra of carotin and xanthophyll, as given by Willstätter and Stoll (1913) [see note b, p. 6] are here reproduced as Fig. 11. It is questionable whether xanthophyll is actually formed by the oxidation of carotin.—*Ed.*

dark, bluish-red solution shows an orange-yellow fluorescence. It crystallizes from salt solutions in hexagonal red crystals.[i]

Phycocyanin,[1] the blue pigment of the blue-green algæ, Cyanophyceæ, is likewise of protein nature; it is soluble in water and glycerine but insoluble in ether and alcohol, its crystals are indigo blue in color.

The brown algæ contain a pigment, *phycophœin*,[2] which is easily soluble in water; in concentrated solutions it is dark reddish-brown.[k]

Engelmann[3] studied the absorption spectra of bright-colored leaves of various plants, and Stahl[4] investigated the biological importance of their coloring.[l]

§5. Influence of Light upon the Decomposition of Carbonic Acid by Plants. An acquaintance with the properties of the different rays of the sun's spectrum (Fig. 12) is prerequisite to an understanding of the researches devoted to this subject. Only the central part of the spectrum, approximately that portion lying between lines *A* and *H*, is visible to the human eye; on either side are invisible rays, infra-red to the left and ultra-violet to the right. Of the visible rays, the yellow are the brighest, the brightness reaching a maximum at line *D* and decreasing to zero beyond *A* and *H*. Brightness does not, however, represent the character of the rays, but only that of the human eye. The energy maximum in the prismatic solar spectrum is usually shown as falling in the region of the infra-red, as in Fig. 12. Nevertheless, recent work upon the distribution of heat in the ordinary diffraction spectrum of sunlight shows the

[1] **Molisch, Hans,** Das Phycocyan, ein krystallisirbarer Eiweisskörper. Bot. Zeitg. **53**: 131–135. 1895.

[2] **Schürt, Franz,** Ueber das Phycophæin. Ber. Deutsch. Bot. Ges. **5**: 259–274. 1887.

[3] **Englemann, Th. W.,** Die Farben bunter Laubblätter und ihre Bedeutung für die Zerlegung der Kohlensäure im Lichte. Bot. Zeitg. **45**: 393–398, 409–419, 425–436, 441–450, 457–469. 1887.

[4] **Stahl, E.,** Ueber bunte Laubblätter. Ein Beitrag zur Pflanzenbiologie. II. Ann. Jard. Bot. Buitenzorg **13**: 137–216. 1896.

[i] On phycoerythrin, see **Haas** and **Hill,** 1921. [See note 3, p. 6.] The best study of this pigment is that of Hanson. (**Hanson, E. K.,** Observations on phycoerythrin, the red pigment of deep-water algæ. New phytol. **8**: 337–344. 1909.)—*Ed.*

[k] But it seems to have been shown that there is no such pigment as phycophæin in the living cells, this being a *post-mortem* product of the decomposition of a colorless chromogen. The brown color of the brown algæ is at least partly due to the presence of carotin. In this connection see the following: **Molisch, Hans,** Das Phycoerythyrin, seine Krystalisirbarkeit und chemische Natur. Bot. Zeitg. **52**: 177–189. 1894. **Idem,** Das Phycocyan ein Krystallisirbarer Eisweisskörper. *Ibid.* **53**: 131–135. 1895. **Idem,** Ueber den braunen Farbstoff der Phæophyceen und Diatomeen. *Ibid.* **63**[1]: 131–144. 1905. **Tswett, M.,** Zur Kenntnis der Phæophyceenfarbstoffe. Ber. Deutsch. Bot. Ges. **24**: 235–244. 1906.—*Ed.*

[l] The *anthocyanins*, or anthocyans, are other pigments that may be mentioned here. They occur very commonly in flowers, leaves, stems, fruits, and even in roots, giving them a red, blue or purple color and frequently masking the green of the chlorophyll in leaves. They are red when acid and blue when alkaline. The color of red apples and many other fruits, of many red, blue and purple flowers, of the beet-root, of red cabbage, of young leaves of many plants, and of the bronze-colored leaves of the copper beech, are due to the presence of these pigments. They are often present along with chlorophyll, as in the case of red cabbage and the copper beech, and still other pigments frequently accompany them. They are soluble in water, alcohol and ether, and the color of the solution alters from red to purple or blue as the reaction is altered from acid to neutral or alkaline. For further information see: **Haas** and **Hill,** 1921. [See note 3, p. 6.] **West, Clarence J.,** Plant pigments: The chemistry of plant pigments other than chlorophyll. Biochem. bull. **4**: 151–160. 1915.—*Ed.*

energy maximum to lie between lines *B* and *C*;[1] and, according to the latest researches, the position of this maximum is not constant but varies from the region of the red to that of the yellow-green, according to the hour of the day. Finally, chemically active or "actinic" rays, with a maximum in the violet region, are frequently differentiated. The term actinic rays really refers to the power of light to decompose silver salts, which is most pronounced in the blue-violet region of the solar spectrum. Many other compounds are decomposed by light, however, frequently in other regions than the blue-violet, and the wave-lengths producing such decomposition are those that are absorbed by the substances decomposed: thus, chlorophyll is most rapidly decomposed by rays between *B* and *C*, exactly the ones most completely absorbed by chlorophyll. Therefore, the curve of chemical intensity, as usually given, has no importance excepting with reference to silver salts: there are no specific "chemical" rays.

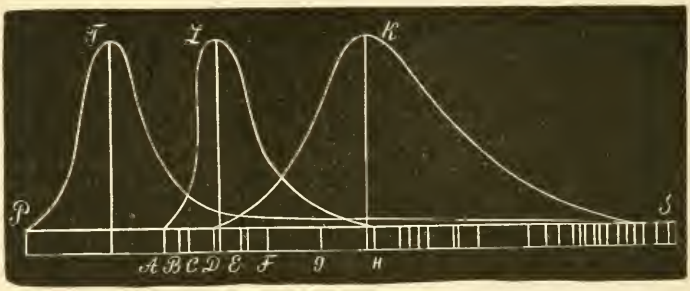


FIG. 12.—Graphs of the prismatic solar spectrum. PA, infra-red; AH, visible; HS, ultra-violet rays; PTS, temperature curve; ALH, curve of light intensity; DKS, curve of effect of light upon the decomposition of silver salts.

Researches upon the influence of light on the decomposition of carbon dioxide and water by plants fall into two groups. One group includes studies dealing with the qualitative side of the question, as to which rays or wave-lengths are most effective in the process. The other includes quantitative investigations, as to how much energy is needed for this decomposition. The first qualitative work was done by Daubeny[m] and Draper[n] the former using

[1] **Langley, [S. P.]**, Observations du spectre solaire. Compt. rend. Paris **95**: 482–487. 1882. **Idem,** Energy and vision. Phil. mag. *V*, **27**: 1–23. 1889. [Sunlight as it reaches plants is so variable in both quality and intensity that each quantitative experiment on photosynthesis, etc., in natural illumination, should be carried out with very careful measurements of solar radiation. Nutting states that the sun's total radiation varies over a range of 8 per cent. of the mean, while the earth's atmosphere, even with a clear sky, absorbs from 20 to 50 per cent., and this varies from minute to minute and from hour to hour of the day. Nutting gives a table (p. 202) of mean solar energy quantities reaching the surface of the earth at Washington at noon, for 26 different wave-lengths, from 385 to 428$\mu\mu$. (See **Nutting, P. G.**, Outlines of applied optics. Philadelphia, 1912.) The wave-length showing the maximum energy value also varies markedly in natural sunlight. For further information see: **Abbot, C. G.,** and **Fowle, F. E., Jr.,** Primary standard pyrheliometer. Ann. Astrophys. Observ. Smithsonian Inst. **2**: 39–47. 1908. **Idem,** The value of the solar constant of radiation. Astrophys. jour. **33**: 191–196. 1911. Also see **Pulling, H. E.,** Sunlight and its measurement. Plant World **22**: 151–171, 187–209. 1919.—*Ed*

[m] **Daubeny, Charles,** On the action of light upon plants, and of plants upon the atmosphere. Phil. trans. Roy. Soc. London **126**: 149–175. 1836.—*Ed.*

[n] **Draper, John W.,** On the decomposition of carbonic acid gas and the alkaline carbonates by the light of the sun. Phil. mag. *III*, **23**: 161–175. 1843. **Idem,** Scientific memoires. 473 p. New York, 1878. P. 184–185.—*Ed.*

light screens and the latter the prismatic spectrum. Both came to the conclusion that plants decompose carbon dioxide most readily under the influence of the yellow light rays. Sachs[1] divided the spectrum into two nearly equal portions, by using a solution of potassium dichromate and one of ammoniacal copper oxide, and found that decomposition of carbon dioxide proceeded almost as energetically in the yellow portion of the spectrum as in direct sunlight, while very little decomposition occurred in the blue-violet region. It is seen, therefore, that it is not the so-called "chemical" rays that are needed for this process, but chiefly the less refrangible rays of the first half of the spectrum. Sachs determined the amount of oxygen given off, using the method of counting ing gas bubbles (Fig. 2).

The next problem was to discover in what rays of the first half of the spectrum the decomposition of carbonic acid was most rapid. The most exact studies upon this point were carried out by Timiriazev,[2] who arranged his experiments as follows: Sunlight was reflected from a heliostat into a dark chamber and was then broken up by a carbon bisulphide prism. Pieces of bamboo leaves were enclosed in glass tubes, with air containing 5 per cent. of carbon dioxide, and these tubes were placed in various regions of the spectrum—in the red between *A* and *B*, in the chlorophyll absorption band between *B* and *C*, in the orange, in the yellow, and in the green. At the conclusion of the experiment analyses of the gas were made, by means of a very sensitive apparatus capable of measuring extremely small amounts of gas. Timiriazev's results are graphically represented in Fig. 13. The ends of the five ordinates, for the five positions in the spectrum where the tubes were exposed, are joined to form a curve, which represents the relative rates of decomposition of carbon dioxide in these different regions of the spectrum. The maximum decomposition occurs in the red, between *B* and *C*, in the region where light is most strongly absorbed by chlorophyll. No decomposition occurs between *A* and *B* (the line *m* represents the amount of carbon dioxide eliminated during the experiment). These results were confirmed by Engelmann[3] and Reinke.[4]

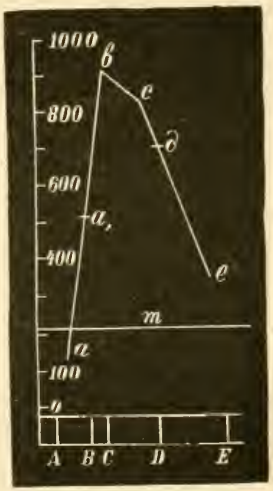


FIG. 13.—Graphs showing relative rates of decomposition of carbon dioxide in different parts of the spectrum. (*After Timiriazev.*)

[1] **Sachs, J.**, Wirkungen farbigen Lichts auf Pflanzen. Bot. Zeitg. 22: 353–358, 361–367, 369–372. 1864.

[2] **Timiriazev, K. A.**, (C.) On the assimilation of light by plants. [Russian.] St. Petersburg. 1875. **Timiriazeff, C.**, Recherches sur la décomposition de l'acide carbonique dans le spectre solaire, par les parties vertes des végétaux. (Extrait d'un Ouvrage "Sur l'assimilation, de la lumière par les végétaux," St.-Pétersbourg, 1875; publié en langue russe.) Ann. chim. et phys. V, 12: 355–396. 1877.

[3] **Engelmann, Th. W.**, Ueber Sauerstoffausscheidung von Pflanzenzellen im Mikrospectrum. Bot. Zeitg. 40: 419–426. 1882.

[4] [**Reinke, J.**, Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoffausscheidung per Pflanzen. II. Die Wirkung der einzelnen Strahlengattungen des Sonnenlichtes. Bot. Zeitg. 42: 17–29, 33–46, 49–59. 1884. See column 27. **Idem**, Die Zerstörung von Chlorophyllösungen durch das Licht und eine neue Methode zur Erzeugung des Normalspectrums. *Ibid.* 43: 65–70, 81–89, 97–101, 113–117, 129–137. 1885. See column 84. **Idem**, Die Abhängigkeit des Ergrünens von der Wellenlänge des Lichts. Sitzungsber (Math.-Naturw. Mitth.), K. Preuss. Akad. Wiss. Berlin. 1893: 301–314. 1893.]

Engelmann was the originator of the bacterial method for the study of photosynthesis. It is well known that many bacteria are active only in the presence of oxygen, and that their movement ceases as soon as there is no oxygen present. If a filament of a green alga is placed in a culture of such bacteria, upon a slide, and if the preparation is protected by a cover glass and darkened, the movement of the bacteria eventually ceases because of lack of oxygen. If a solar spectrum is now projected upon the alga filament, under the microscope, it is seen that the movement of the bacteria is renewed in the neighborhood of both of the main chlorophyll absorption bands (Fig. 14), being especially pronounced in the red and appreciably weaker in the blue. It is only in the spectral regions thus undicated, therefore, that an evolution of oxygen occurs, to which the bacteria respond.

The degree of difference between the efficiences of the blue and red spectral regions was established by Timiriazev.[1] For this purpose he divided the

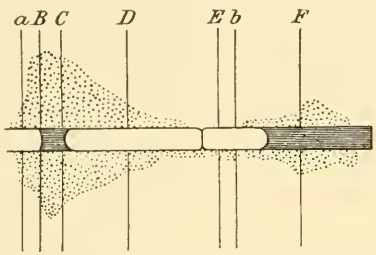


FIG. 14.—Bacterial movement in the regions of the absorption bands of chlorophyll. (*After Englemann.*) The dots indicate moving bacteria and the letters denote the Fraunhofer lines.

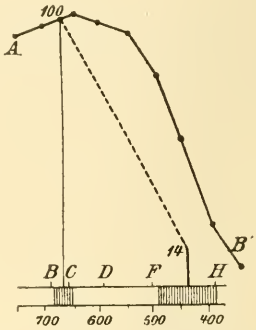


FIG. 15.—*AB*, distribution of heat energy in the solar spectrum. (*After Langley.*) 100–14, relative rates of carbon-dioxide decomposition by leaves in red and in blue light.

spectrum into two equal parts by means of a cylindrical lens and a prism with a very small angle of refraction. Flat-sided glass tubes containing pieces of leaves of equal area were placed in the bright bands of blue and yellow light thus obtained, and a gas analysis of the tube contents was made after three-quarters of an hour or an hour. If the intensity of carbon dioxide decomposition in the less refrangible (red-yellow) light be taken as 100, then the corresponding intensity in the more refrangible (blue) light is 54. Thus the light absorbed by the leaves in the blue half of the spectrum is only about half as effective as that absorbed in the other half. The absorption spectrum of the leaves used in Timiriazev's experiment is presented in Fig. 15. It must be noted, however, that the two absorption bands are not of equal width, the one in the blue-violet region of the normal spectrum being more than three times as wide as the band between *B* and *C*. If each of the ratios mentioned above is

[1] **Timiriazev, C.,** Photochemische Wirkung der am Rande des sichtbaren Spektrums liegenden Strahlen. 1893. (Russian.)*

divided by the breadth of the corresponding effective absorption band, there is obtained for an average wave-length of the red region, 100, and for a similar average in the blue-violet, 14, a relation which is graphically represented in Fig. 15. Thus red light is relatively much more effective than blue-violet light. How can this difference be explained? Obviously the explanation is to be found in a consideration of the energy of the different wave-lengths expressed in terms of their respective heat values, and (as will be seen from comparison of the curve of decomposition of carbon dioxide with the Langley curve, *AB*, representing the heating effect of the various parts of the solar spectrum) both of these increase in the same direction. So the blue and violet rays have only a comparatively slight effect in the decomposition of carbon dioxide, because, even though they are absorbed by chlorophyll, they represent only a very small amount of energy.°

The dependence of the process of decomposition of carbon dioxide upon the energy of the light rays was demonstrated in a still more detailed manner by the experiments of Rikhter.[1] Only light that is absorbed can decompose carbon dioxide, and those wave-lengths of the absorbed light are most effective which furnish the greatest amount of heat energy. Rikhter used solutions of potassium dichormate, ammoniacal copper oxide and potassium permanganate as light filters. The plant received the following relative amounts of light when placed behind the various filters:

Water	Potassium Dichromate Solution	Ammoniacal Copper Oxide Solution	Potassium Permanganate Solution
1000	491	177	233.0
	100	36	47.5

The corresponding relative rates of carbon dioxide decomposition behind the same light screens proved to be, on the average, as follows:

Water	Potassium Dichromate Solution	Ammoniacal Copper Oxide Solution	Potassium Permanganate Solution
1000	494	168.0	249
	100	34.4	48

The numbers in the two series agree so closely as to suggest that the amount of photosynthetic work accomplished by a ray of light is proportional to the amount of energy absorbed by the leaf, and is independent of the wave length of the ray and of its position in the spectrum.[2]

[1] **Richter, Andrè,** Étude sur la photosynthèse, et sur l'absorption par la feuille verte des rayons de différentes longueurs d'onde. Rev. gén. bot. 14: 151–169. 211–218. 1902. **Kohl,** 1897. [See p. 5, note 1.]

[2] See also: **Kniep, H.,** and **Minder, F.,** Ueber den Einfluss verschiedenfarbigen Lichtes auf die Kohlensäureassimilation. Zeitsch. Bot. 1: 619–650. 1909. [**Puriewitsch, K.,** Untersuchungen über Photosynthese. Jahrb. wiss. Bot. 53: 210–254. 1913.]

° These statements apply to leaves and should not be interpreted as necessarily applying to chlorophyll, for leaves contain carotin, etc., which surely affect their power to absorb radiation. Some referencess on sunlight have been given in note 1, p. 22. See also: **Iwanowski, D.,** Ein Beitrag zur physiologischen Theorie des Chlorophylls. Ber. Deutsch. Bot. Ges. **32:** 433–447. 1914.—*Ed.*

Carbon dioxide is thus seen to be decomposed most rapidly in green plants by the light rays between lines *B* and *C*. But when other pigments besides chlorophyll are present, the maximum of this decomposition may fall in another part of the spectrum.[1] In the Cyanophyceæ the maxumim occurs at *D*; the brown algæ show a maximum between *D* and *E*, although the decomposition between *B* and *C* is here almost as great; finally, the red algæ have a maximum between *D* and *E* also, but the decomposition between *B* and *C* is here very weak. These facts are in agreement with the distribution of the various algæ, according to depth, in the ocean; while the surface layer of water is mainly inhabited by green algæ, the red forms are found at very great depths. Spectroscopic investigations have shown that red light, which is essential to green algæ, is quickly absorbed by water and that this light is entirely absent at no great distance below the surface. On the other hand, the green and blue rays, which are absorbed by the red algæ, attain great depths.

According to Engelmann,[2] plants that contain no chlorophyll may also decompose carbon dioxide, provided they contain another pigment; as, for instance, the purple bacteria.[p]

Engelmann's theory of complementary pigments found confirmation in the interesting researches of Gaidukov[3] upon the influence of colored light upon the color of *Oscillaria*. This alga tends to assume the color complementary to that of the light acting upon it, and the longer the organism remains in the colored light the more pronounced is the response. The following kinds of illumination produced the following colorations in the organism.

Color of Light	Color of Alga
Red	Green
Brownish-yellow	Blue-green
Green	Reddish
Blue	Brownish-yellow

The principle illustrated by this phenomenon was designated by Gaidukov as the law of *complementary chromatic adaptation*.

The amount of light[4] necessary for the decomposition of carbon dioxide is

[1] **Engelmann, Th. W.,** Farbe und Assimilation. Bot. Zeitg. **41**: 1–13, 17–29. 1883.

[2] **Engelmann, Th. W.,** Die Purpurbacterien und ihre Beziehungen zum Licht. Bot. Zeitg. **46**: 661–669, 677–689, 693–710, 709–720. 1888.

[3] **Gaidukov, N.,** Ueber den Einfluss farbigen Lichts auf die Färbung lebender Oscillarien. Abh. K. Preuss. Akad. Wiss. Berlin, 1902. Anhang, Phys. Abh. V., p. 1–36.

[4] **Kreusler, U.,** Ueber eine Methode zur Beobachtung der Assimilation und Athmung der Pflanzen und über einige diese Vorgänge beeinflussende Momente. Landw. Jahrb. **14**: 913–965. 1885. **Timriazeff, C.,** Sur le rapport entre l'intensité des radiations solaires et la décomposition de l'acide carbonique par les végétaux. Compt. rend. Paris **109**: 379–382. 1889. **Pantanelli, Enrico,** Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen. Jahrb. wiss. Bot. **39**: 167–228. 1904. **Lubimenko, W.,** Sur la sensibilité de l' appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. gén. Bot. **17**: 381–415. 1915. **Idem,** concentration du pigment vert et l'assimilation chlorophyllienne. *Ibid.* **20**: 162–177, 217–238, 253–267, 285–297. 1908. **Idem,** Production de la substance sèche et de la chlorophylle chez les végétaux supérieurs aux différentes intensités lumineuses. Ann. sci. nat. Bot. *IX*, **7**: 321–415. 1908.

[p] But Molisch's studies indicate that the purple bacteria are not capable of the photosynthesis of carbohydrates from carbon dioxide and water. See: **Molisch, Hans,** Die Purpurbakterien nach neuen Untersuchungen, eine mikrobiologische Studie. 92 p. Jena, 1907. (A misstatement occurred here in the first printing.)—*Ed.*

closely related to the individual properties of the plant, some forms needing more and other less light. Trees were long ago differentiated by students of forestry into two types, heliophobous (shade plants) and heliophilous (non-shade plants); among the first are included, for example, Abies (fir), Taxus (yew), Fagus (beech), Tilia (linden); among the latter, Pinus (pine), Larix (larch), Betula (birch), Robina (locust).

Schistostega osmundacea, a moss that grows in dark caves, may be mentioned as an example of plants that can thrive in extremely weak light. Its protonema has a very peculiar structure (Fig. 16), and, although existing in semi-darkness, it appears emerald green. Single filaments of the protonema, as they grow upward, each form a plate of cells lying at right angles to the direction of the impinging light. Each cell of this plate has the form of a lens and the chloroplasts lie in the prolonged basal region. Acting like biconvex lenses, these cells concentrate the light of the half-dark cave sufficiently to allow carbon

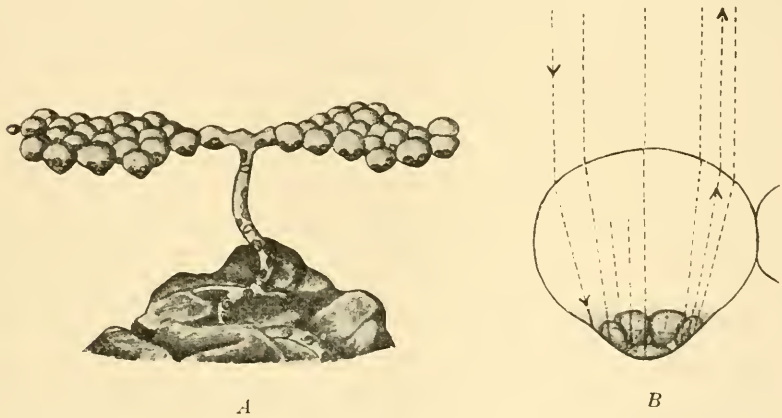


FIG. 16.—*Schistostega osmundacea: A*, protonema; *B*, diagram representing the path taken by rays of light as they enter and leave the cells of the protonema.

dioxide decomposition by the chloroplasts. A part of the light is reflected, thus rendering the protonema luminous.

In general, plants are adapted to the minimum of available light (Wiesner, Liubimenko). In heliophilous plants (which thrive best in bright sunshine) the rate of carbon dioxide decomposition increases continuously with increase in light intensity;[q] on the other hand, for heliophobous plants (which thrive in shade or in regions of low light intensity) there exists an optimum light intensity, and any increase beyond this optimum results in a decrease in the amount of carbon dioxide decomposed. This difference is related to the different amounts of chlorophyll contained in the two kinds of plants. Liubimenko was able to show that heliophobous plants are richer in chlorophyll than are heliophilous ones. Within limits, the greater the amount of light

[q] It is not to be understood that there are no optimum light intensities for carbon-dioxide decomposition in plants that grow best in bright sunshine, only that such optima are markedly higher than those for plants that grow best in shade.—*Ed.*

and the higher the temperature, the smaller is the amount of chlorophyll formed by the plant.

§6. **Products of Photosynthesis.**[1]—The simplest equation that may represent the exchange of gases in photosynthesis is $CO_2 = C + O_2$. The carbon is retained by the plant, combined with other elements in the form of organic substances. The question now arises as to what are to be considered as the first products of photosynthesis. The investigations of Sachs[2] showed that the first *visible* product is starch. If leaves are kept for several days in darkness the starch completely disappears from the chlorophyll bodies, and if the leaves are then returned to light starch soon appears again. Small traces of starch may be recognized by the method of Böhm, whereby leaves are first decolorized by alcohol and then treated with caustic potash and iodine solution; the starch grains, greatly swollen by potassium hydroxide, are stained by iodine and thus become visible. If a part of the leaf is covered with tinfoil before it is exposed to light, and if, after the exposure, the leaf is decolorized with alcohol and then treated with iodine, the portion that was shaded becomes yellowish brown, while the rest of the leaf is blue or black, according to the amount of starch present (Fig. 17). The experiment becomes particularly striking if the whole leaf is covered with a piece of tinfoil, or cardboard, from which the letters of the word starch, etc., have been cut out as in a stencil; after the treatment described above, the letters stand out blue against a brown background.[r]

FIG. 17.—Accumulation of starch in the illuminated portion of a leaf. The light-colored portion was shaded by tinfoil and the starch has been stained by iodine.

According to Famintsyn,[3] algæ may be very satisfactorily employed in this connection; the presence of starch may be shown after only half an hour's illumination from a bright lamp. According to Kraus,[4] algæ may form starch in sunlight within a period of five minutes. As Godlewski[5] has shown, starch

[1] **Brown, H. T.,** and **Morris, G. H.,** A contribution to the chemistry and physiology of foliage leaves. Jour. Chem. Soc. London **63**: 604–677. 1893.

[2] **Sachs, J.,** Ueber den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern. Bot. Zeitg. **20**: 365–373. 1862. **Idem,** Ueber die Auflösung und Wiederbildung des Amylums in den Chlorophyllkörnern bei wechselnder Beleuchtung. *Ibid.* **22**: 289–294. 1864.

[3] [**Famintzin, A.,** Die Wirkung des Lichtes auf Algen und einige andere ihnen nahe verwandte Organismen. Jahrb. wiss. Bot. **6**: 1–44. 1867. See P. 34.]

[4] [**Kraus, Gregor,** Einige Beobachtungen über den Einfluss des Lichts und der Wärme aud die Stärkeerzeugung im Chlorophyll. Jahrb. wiss Bot. **7**: 511–531. 1868.]

[5] **Godlewski, Emil,** Abhängigkeit der Stärkebildung in den Chlorophyllkörnern von dem Kohlensäuregehalt der Luft. Flora, n. R. **31**: 378–383. 1873.

[r] The experiment should be performed in such manner that access of the carbon dioxide of the air to the stomata is clearly not hindered; otherwise the conclusion given is not logically substantiated. (See **Ganong, W. F.,** A laboratory course in plant physiology. 2 ed., New York, 1908. P. 86–90.) It is usually best to transfer the decolorized leaves from alcohol to water, then to an aqueous solution of potassium hydroxide, after which an aqueous solution of potassium iodide and iodine is added to bring out the color reaction. The iodine solution may be prepared by dissolving 5 g. of the iodide in water, then dissolving 1 g. of iodine in this, and diluting the resulting double solution to a volume of 1000 cc. or less.—*Ed.*

can be formed in light only in the presence of carbon dioxide. In a closed chamber, illuminated but free from this gas, no starch was formed; indeed, if starch had been originally present its amount decreased under these conditions. The chloroplasts of some plants do not form starch at all, as is the case with laves of *Allium cepa* (onion), *A. fistulosum*, *Asphodelus luteus*, *Orchis militaris*, and *Lactuca sativa* (lettuce), but in all these instances glucose is formed instead of starch.

According to whether starch ($(C_6H_{10}O_5)_n$) or glucose ($C_6H_{12}O_6$) is considered as the first product of photosynthesis, the chemical equation representing the process may take one or the other of the two forms given below:

$$(1) \qquad 6\ CO_2 + 5\ H_2O = C_6H_{10}O_5 + 6\ O_2.$$
$$(2) \qquad 6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2.$$

Timiriazev[1] showed by direct experiment that the formation of starch in light is brought about by the same rays of the spectrum as are effective in the decomposition of carbon dioxide. By means of a heliostat, a spectrum was thrown upon a leaf of a plant that had been previously exposed to darkness so as to free the leaves of starch; two strips of paper were fastened across the leaf with the spectrum falling between them, and upon these strips were recorded the positions of the Fraunhofer lines in the spectrum. At the end of the experiment, after the leaf had been decolorized by alcohol and stained with iodine, it became evident that starch formation had occurred exactly in the regions corresponding to the absorption bands of chlorophyll. In such an experiment the band between lines *B* and *C* is especially pronounced, and a fainter iodine-starch color is noticeable in the orange-yellow region, this coloration gradually decreasing in intensity and ceasing not far beyond the *D* line. Thus starch is produced by those wave-lengths of light that cause the decomposition of carbon dioxide, the rays between *B* and *C* being most effective in both cases.

Briosi[2] was unable to find starch in the leaves of Musa (banana) and Strelitzia, but found oil instead, and expressed the opinion that the latter was the first product of photosynthesis in these plants. Holle[3] and Godlewski[4] were able to prove, however, that this supposition is untenable.

Baeyer[5] advanced the hypothesis that formaldehyde is really the first product of photosynthesis, and that carbohydrates arise from this by progressive condensation or polymerization. The formation of formaldehyde thus supposed is represented by the equation, $CO_2 + H_2O = CH_2O + O_2$. Baeyer based his supposition upon a discovery by Butlerow[6] that oxymethylene ($C_3H_6O_3$) is con-

[1] **Timiriazeff, C.,** Enregistrement photographique de la fonction chlorophyllienne par la plante vivante. Compt. rend. Paris **110**: 1346–1347. 1890.

[2] [**Briosi, Giovani,** Ueber normale Bildung von Fettartiger Substanz im Chlorophyll. Bot. Zeitg. **31**: 529–533, 545–550. 1873.]

[3] **Holle, H. G.,** Ueber die Assimilationsthätigkeit von *Strelitzia reginæ*. Flora, n. R. **35**: 113–120, 154–160, 161–168, 184–192. 1877.

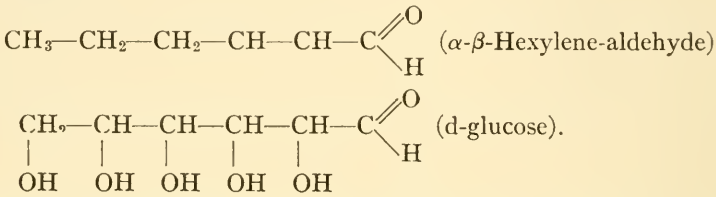
[4] **Godlewski, Emil,** Ist das Assimilationsprodukt der Musaceen Oel oder Stärke? Flora, n. R. **35**: 215–220. 1877.

[5] **Baeyer, Adolf,** Ueber die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung. Ber. Deutsch. Chem. Ges. **3**: 63–75. 1870.

[6] [**Butlerow, A.,** Bildung einiger Zuckerarten durch Synthese. Liebig's Ann. Chem. u. Pharm. **120**: 295–298. 1861. **Idem,** Formation synthétique d'une substance sucrée. Compt. rend. Paris **53**: 145–147. 1861.]

verted into a sugar-like substance in the presence of calcium and barium hydroxides.

Reinke is of the opinion that the hydrate of carbonic acid and not the anhydride, is decomposed in the light, as indicated by the equation, $H_2CO_3 = CH_2O + O_2$. The same author[1] was successful in showing that substances possessing aldehyde characters generally occur in green plants, and Curtius and Reinke[2] succeeded in isolating a material of this sort and in identifying it chemically. Curtius and Franzen[3] isolated α-β-hexylene-aldehyde from the leaves of Carpinus (horn-beam). This aldehyde shows the same carbon skeleton as does glucose, as becomes evident from a comparison of their structural formulæ:



Pollacci[4] found, furthermore, that the green parts of plants gave a positive aldehyde reaction with Schiff's reagent only if they had been previously exposed to light and carbon dioxide; if the plants had previously been deprived of both light and this gas they gave, as did also fungi, no reaction for aldehyde.[a]

Formaldehyde can be utilized by green plants in the formation of carbohydrates, but none is absorbed in darkness.[5]

Walther Löb's[6] interesting researches have furnished experimental evidence in favor of Baeyer's hypothesis. He used a silent electric discharge as source of energy, instead of sunlight, and established the following principal reactions between carbon dioxide and water, etc.

1. $2\ CO_2 = 2\ CO + O_2$
2. $CO + H_2O = CO_2 + H_2$
3. $H_2 + CO = H_2CO$
4. $CO + H_2O = HCOOH$
5. $3\ O_2 = 2O_3$
6. $2\ H_2 + 2O_3 = 2\ H_2O_2 + O_2$

[1] **Reinke, J.**, Studien über das Protoplasma. I–III. Untersuch. Bot. Lab. Göttingen **2**: 1–202. 1881. **Idem**, Studien über das Protoplasma. 2te Folge. *Ibid.* **3**: 1–76. 1883.

[2] **Curtius, Theodor**, and **Reinke, J.**, Die flüchtige, reducirende Substanz der grünen Pflanzentheile. Ber. Deutsch. Bot. Ges. **15**: 201–210. 1897.

[3] **Curtius, Theodor**, and **Franzen, Hartwig**, Aldehyde aus grünen Pflanzenteilen. I. Mitteilung. Ueber α-β-Hexylenaldehyd. Sitzungsber. (math.-naturw. Kl.) Heidelberg. Akad. Wiss. Jahrgang 1910, Abhandl. 20. 13 p. 1910.

[4] **Pollacci, Gino**, Intorno all' assimilazione clorofilliana delle plante. Atti Ist. Bot. Univ. Pavia *II*, **7**: 1–21. 1902. On the synthesis of carbohydrates in chloroplasts see: **Fischer, Emil**, Synthesen in der Zuckergruppe. II. Ber. Deutsch. Chem. Ges. 27^{III}: 3189–3232. 1894. P. 3230.

[5] **Grafe, Viktor**, Untersuchungen über das Verhalten grüner Pflanzen zu gasförmigen Formaldehyd. II. Ber. Deutsch. Bot. Ges. **29**: 19–26. 1911. **Idem**, Die biochemische Seite der Kohlensäure-Assimilation durch die grüne Pflanze. Biochem. Zeitsch. **32**: 114–129. 1911. [**Baker, Sarah M.**, Quantitative experiments on the effect of formaldehyde upon living plants. Ann. bot. **27**: 411–442. 1913.]

[6] **Löb, Walther**, Zur Kenntnis der Assimilation der Kohlensäure. Landw. Jahrb. **35**: 541–578. 1906.

[a] On reactions for identifying formaldehyde in plant parts, see **Haas** and **Hill**, 1921. [See note 3, p. 6.]—*Ed.*

The formation of formaldehyde was limited by the last three (secondary) reactions; hydrogen combined more easily with oxygen, to form hydrogen peroxide, than with carbon monoxide. To obtain formaldehyde in greater quantity Löb added a reducing agent (salicylic aldehyde, pyrogallol or chlorophyll. Glycolic aldehyde (which represents the simplest sugar), as well as formic acid and formaldehyde, arises from the action of the silent discharge upon carbon monoxide, water, and hydrogen; $2(H_2 + CO) = CH_2OH - CHO$ (glycolic aldehyde). By the concentration of its solution *in vacuo* this substance is readily transformed into a tetrose or hexose.[1]

Stoklasa and Zdobnický[2] found that formaldehyde was formed by the action of ultra-violet light upon water vapor and carbon dioxide in the presence of potassium hydroxide, but no carbohydrates were thus produced. Sugar was formed, however, under these same conditions, when hydrogen was present in the nascent state.[t]

Sorbose is formed by the action of light upon a mixture of formaldehyde and oxalic acid.[3]

Bonnier and Mangin, as has already been mentioned (see page 4), have shown that if the interchange of gases accompanying the process of photosynthesis is determined independently of respiration, the ratio $\frac{CO_2}{O_2}$ is found to be somewhat less than unity. From this we must suppose that substances other than carbohydrates and less easily oxidized than these, are formed in the leaves under the influence of sunlight. The supposition that proteins also arise in the process of photosynthesis has been frequently advanced. This is supported by the quantitative researches of Sapozhnikov,[4] in which he established the fact that an increase in protein occurs parallel with the accumulation of carbohydrates in light. Posternak[5] is of the opinion that oxymethyl-phosphoric acid is also formed in leaves in the presence of light.

[1] **Bach, A.**, Sur l'evolution biochimique du carbone. Arch. sci. phys. et nat. **5**: 401–415, 520–535 1898. This deals with the theory of photosynthesis.

[2] **Stoklasa, J.**, and **Zdobnický, W.**, Photochemische Synthese der Kohlenhydrate aus Kohlensäureanhydrid und Wasserstoff in Abwesenheit von Chlorophyll. Biochem. Zeitsch. **30**: 433–456. 1911.

[3] **Inghilleri, Giuseppe**, Photochemische Synthese der Kohlenhydrate. I. Mitteilung. Bildung von Sorbose. Zeitsch. physiol. Chem. **71**: 105–109. 1911.

[4] **Saposchnikoff, W.**, Bildung und Wanderung der Kohlenhydrate in den Laubblättern. Ber. Deutsch. Bot. Ges. **8**: 233–242. 1890. **Idem**, Beitrag zur Kenntniss der Grenzen der Anhäufung von Kohlenhydraten in den Blättern. *Ibid.* **11**: 391–393. 1893. **Idem**, Eiweissstoffe und Kohlenhydrate der grünen Blätter als Assimilations-producte. 61 p. Tomsk, 1894. [Russian.] [Rev. by **Rothert** in: Bot. Centralbl. **63**: 246–251. 1895.

[5] **Posternak, S.**, Contribution à l'étude chimique de l'assimilation chlorophyllienne. Sur le premier produit d'organization de l'acide phosphorique dans les plantes à chlorophylle avec quelques remarques sur le rôle physiologique de l'inosite. Rev. gén. bot. **12**: 5–24, 65–73. 1900.

[t] Further, on the artificial formation of formaldehyde, etc., from carbon dioxide and water, see: **Berthelot, D.**, and **Gaudichon, H.**, Synthèse photochimique des hydrates de carbone aux depens des éléments de l'anhydride carbonique et de la vapeur de l'eau, en l'absence de chlorophylle; synthèse photochimique des composés quartenaires. Compt. rend. Paris **150**: 1690–1693. 1910. For a review of this general subject, see: **Spoehr, H. A.**, Theories of photosynthesis. Plant world **19**: 1–16. 1916. It should be remembered that the reactions that take place in leaves may not be the same as those studied *in vitro*. Very little experimental work has been done on the photochemical changes to which chlorophyll itself is subject.—*Ed.*

According to Krasheninnikov[1] a definite relation holds between the amount of carbon dioxide decomposed and the concomitant increase in dry weight, as is evident from the following average values: for a square meter of leaf surface the amount of carbon dioxide decomposed was 2286 cc. or 4.48 g., while the corresponding increase in dry weight was 2.94 g. The increase in dry weight for each weight unit of carbon dioxide decomposed was found to have the values given below, for the different plant forms considered.

Bamboo	0.60
Cherry-laurel	0.60
Sugar cane	0.67
Linden	0.74
Tobacco	0.68

It is seen that this ratio appears to be fairly constant. The formation of a carbohydrate with the composition $C_{12}H_{22}O_{11}$ (like cane sugar) would give this ratio a value of 0.64.

Investigations upon the first products of photosynthesis agree with plant analyses in showing that an assimilation of water occurs simultaneously with that of carbon dioxide. In every green plant the formation of organic substance in sunlight is accompanied by assimilation of carbon, hydrogen and oxygen. The bulk of the dry weight of the plant is due to these three elements; this dry weight is made up of about 45 per cent. carbon, 42 per cent. oxygen, 6.5 per cent. hydrogen, 1.5 per cent. nitrogen, and 5 per cent. mineral constituents. Thus plants obtain more than 90 per cent. of their dry weight from the carbon dioxide of the air and the water of the soil.

§7. Assimilation of Solar Radiant Energy by Green Plants.—We have already seen that green plants are able, with absorption of sunlight, to build up combustible organic compounds out of non-combustible inorganic substances. The chloroplasts of green plants furnish conditions for this process. Animal heat and movement, the heat of fuels, the work of steam engines, are all due to the freeing of the radiant energy of the sun which was previously fixed by the chloroplasts.

Julius Robert Mayer stated very clearly the rôle of green plants when he said:

> Nature has set for herself the task of seizing the sunlight in its flight, as it streams upon the earth, and of accumulating the most swiftly moving of all forms of energy by transforming it into a potential state. To accomplish this purpose she has covered the surface of the earth with living organisms that absorb sunlight into themselves and thus generate a permanent store of potential chemical energy. These organisms are plants, and the plant world forms a reservoir in which the fleeting rays of light are caught and cleverly hoarded for future use.[2]

The following interesting anecdote is taken from the biography of the engineer Stephenson, and shows that he also was well acquainted with this rôle played by plants.

> On Sunday as people were returning from church, with Stephenson and Buckland among

[1] Kraschéninnikoff, Th., Ansammlung der Sonnenengergie in den Pflanzen. Moskow, 1901. [Russian.]*

[2] Mayer, Julius Robert, Die Mechanik der Wärme. P. 34. Leipzig, 1911. (Ostwald's Klassiker no. 180.)

them, the whole company stopped upon the terrace beside Drayton Castle to watch a railway train as it vanished rapidly in the distance, with a trail of white smoke behind it.

"Well, Buckland," said Stephenson as he turned to the famous geologist, "Answer me a question, not a very easy one, perhaps. Can you tell me what sort of force it is that drives yonder train along?"

"Well," answered the geologist, "I should think that the force was one of your great engines."

"Yes but what moves the engine?"

"Why, one of your Newcastle engineers, of course."

"No, sunlight."

"How can that be?" asked the doctor.

"I assure you it is nothing else," replied the engineer. "It is light that has lain stored in the earth for many thousands of years; the light absorbed by the plant during its growth is essential for the condensation of carbon, and this light, which has been buried in the coal measures for so many years, is now unearthed and, being freed again as in this locomotive, serves great human ends."[1]

Along with the accumulation of starch there occurs also a storage of potential energy in the plant. Krasheninnikov[2] was able to demonstrate this relation by direct experiment. Half-leaves were removed from the plant and their areas were measured, after which they were dried and burned, to determine the heat of combustion of their dry substance. The remaining half-leaves, also removed from the plant but still alive, were exposed to light for a time, and the amount of carbon dioxide which they decomposed was measured. They were then dried and their heat of combustion was also determined. Below are given the average values of all the determinations, calculated for an area of 1 sq. m. of leaf surface exposed to the light.

Increase in dry weight	3.51 g.
Increase in carbohydrates	2.46 g.
Increase in carbon	1.58 g.
Increase in heat of combustion	15,350 g.-cal.
Amount of carbon dioxide decomposed	5.626 g.

From the data of this experiment Krasheninnikov calculated that there was an increase of from 2.2 to 3.6. g.-cal. for each gram of carbon dioxide decomposed.[u]

It is also desirable to know what proportion of the radiant energy falling upon the leaf is assimilated. The first calculation bearing upon this question was made by Becquerel,[3] with the following results, which represent the yearly amounts of assimilation for three different types of vegetation, per hectare (2.5 acres).

[1] **Mayer, Adolf Eduard,** Lehrbuch der Agrikulturchemie. 5 Aufl. Heidelberg, 1901–1902. P. 35.

[2] **Kraschéninnikoff,** 1901. [See note 1, p. 32.]

[3] **Becquerel, Alexandre E.,** La lumière, ses causes et ses effects. Paris, 1867–1868.

[u] On alterations in the areas of leaves when the latter are transferred from shade to sunlight, which may possibly have some influence on the magnitudes of such values as these, see: **Thoday, D.,** Experimental researches on vegetable assimilation and respiration. V. A critical examination of Sachs' method for using increase of dry weight as a measure of carbon dioxide assimilation in leaves. Proc. Roy. Soc. London **B82**: 1–55. 1909.—*Ed.*

Kind of Vegetation	Kilograms of Carbon Assimilated per Hectare
Forest in Central Europe	1800
Well fertilized meadow	3500
Helianthus tuberosus (Jerusalem artichoke)	6000

From a series of calculations, Becquerel came to the conclusion that, in France, plants assimilate less than 1 per cent. of the radiant energy that reaches them. Timiriazev arrived at the same result, and Brown's[1] more recent determinations give a still smaller value. In the latter case a Helianthus leaf received on a sunny day 600,000 g.-cal. per square meter of leaf surface per hour. In the same time an equal surface of leaf produced 0.8 g. of carbohydrates, for the formation of which 3200 g.-cal. were necessary. Thus the leaf accumulated, by the photosynthetic process, barely 0.5 per cent. of the solar energy reaching it; viewed as a machine designed to produce organic compounds, its efficiency is thus seen to be far from high.[v]

An excess of light has a retarding effect upon increase in dry weight. It appears that different rays of the spectrum are effective in different stages of the photosynthetic process.[2]

The importance of light to plants is not confined to the photosynthesis of carbohydrate from carbon dioxide and water; light is necessary for very many kinds of chemical reactions taking place in plants. Among the investigations that already testify to this are those upon the influence of light in protein formation. Numerous other reactions that are influenced by light and that are purely chemical in nature furnish additional evidence upon this point. Ciamician and Silber[3] were able to establish the fact that very many oxidations, reductions, hydrolyses, polymerizations and condensations are effected by light; such changes may progress very rapidly when an inorganic substance is involved.[4]

§8. Influence of External and Internal Conditions upon Photosynthesis.—One of the most important of the external conditions upon which various physiological processes depend is the temperature of the surroundings. The influence of temperature upon the velocity of the greening process has been shown above. Photosynthesis, on the other hand, is only very slightly affected

[1] **Brown, H. T.,** Recherches sur la fixation du carbone par les feuilles et sur la diffusion de l'acide carbonique. Traduit librement de l'Anglais par M. E. Demoussy. Ann. agron. **27**: 428–438. 1901. [The original paper is: **Brown, Horace T.,** Opening address by the President of Section B (Chemistry), Brit. Assoc. Adv. Sci., Nature **60**: 474–483. 1899. (See also correction: *ibid.* **60**: 544. 1899.) Also published in: Rept. Brit. Assoc. Adv. Sci. **1899**: 664–683. 1900. See also: **Brown, H. T.,** and **Escombe F.,** Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. trans. Roy. Soc. London **B193**: 223–292. 1900.]

[2] **Liubimenko, V. N.,** La quantité de pigment vert dans le grain de chlorophylle et l'energie de la photosynthese. [Abstract in French, p. 263–266; text in Russian.] Trav. Soc. Imp. Nat. St.-Pétersbourg Ser. *III*, Sect. Bot. **41**: 1–266. 1910.

[3] **Ciamician, G.,** Sur les actions chimiques de la lumière. Bull. Soc. chim. France 4 (fasc. 15): i–xxvii. 1908. [A special appendix to this fasc., bound at end of vol., separately paged.] [See also note 1, p. 180.]

[4] **Neuberg, Carl,** Chemische Umwandlungen durch Strahlenarten. I. Mitteilung. Katalytische Reaktionen des Sonnenlichtes. Biochem. Zeitsch. **13**: 305–320. 1908. **Idem,** Ueber die Reaktion der Gallensäuren mit Rhamnose bzw. δ-Methyl-furfurol. *Ibid.* **14**: 349–350. 1908. **Idem,** Bemerkung über die "Glucothionsäuren." *Ibid.* **16**: 250–253. 1909. **Idem,** Notiz über Phytin. *Ibid.* **16**: 406–410. 1909.

[v] In such calculations as this it is to be noted that the plant does not absorb nearly all the energy reaching it and that all the organic material formed does not appear in the final determinations.—*Ed.*

by temperature. According to the investigations of Kreusler,[1] the decomposition of carbon dioxide begins at temperatures almost as low as the freezing point and continues up to 50°C. His data are presented below.

Temperature *Deg. C.*	Amount of CO_2 Decomposed	Temperature *Deg. C.*	Amount of CO_2 Decomposed	Temperature *Deg. C.*	Amount of CO_2 Decomposed
2.3	1.0	20.6	2.6	37.3	2.3
7.5	1.7	25.0	2.9	41.7	2.0
11.3	2.4	29.3	2.4	46.6	1.3
15.8	2.8	33.0	2.4		

If the amount of carbon dioxide decomposed in a unit of time at 2.3° be represented by unity it is seen that this rate is not yet equal to 3 at 25°. Such a rise of temperature increases the rate of respiration to many times its original value.[w]

Great fluctuations in atmospheric pressure exert a marked influence upon photosynthesis.[2]

The process of photosynthesis is dependent upon the amount of chlorophyll present in the leaves.[3] The anatomical structure of these organs is also of importance, the stomata playing a particularly pronounced rôle. Mangin[4]

[1] **Kreusler, U.,** Beobachtungen über die Kohlensäure-Aufnahme und -Ausgabe (Assimilation und Athmung) der Pflanzen. II. Mittheilung: Abhängigkeit vom Entwicklungszustand—Einfluss der Temperatur. Landw. Jahrb. **16**: 711–755. 1887. [**Idem,** same title. III. Mittheilung: Einfluss der Temperatur; untere Grenze der Wirkung. *Ibid.* **17**: 161–175. 1888. **Idem,** Beobachtungen über Assimilation und Athmung der Pflanzen. IV. Mittheilung: Verhalten bei höheren Temperaturen; Kohlensäure-ausscheidung seitens getödterer Exemplare; Kohlensäure Verbrauch, wenn Ober- und Unterseite der Blätter dem Licht Zugewendet. *Ibid.* **19**: 649–668. 1890.]

[2] **Friedel, Jean,** L'assimilation chlorophyllienne aux pressions inférieures à la pression atmosphèrique. Rev. gén. bot. **14**: 337–355, 369–390. 1902.

[3] **Liubimenko,** 1910. [See note 2, p. 34.]

[4] **Mangin, L.,** Sur le rôle des stomates dans l'entrée ou la sortie des gaz. Compt. rend. Paris **105**: 879–881. 1887.

[w] But Gabrielle Matthaei's very careful studies (**Matthaei, Gabrielle L. C.,** Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxid assimilation. Phil. trans. Roy. Soc. London **B197**: 47–105. 1905) show that the influence of temperature upon photosynthesis in leaves of *Prunus laurocerasus* (cherry-laurel) is much more pronounced than is indicated by Kreusler's numbers. Her results are shown below, the amounts representing hourly rates per 50 sq. cm. of leaf.

Temperature, *deg. C.*,	−6	8.8	11.4	15	23.7	30.5	37.5	40.5	43.0
CO_2 assimilated, *g.*	0.0002	0.0038	0.0048	0.0070	0.0102	0.0157	0.0238	0.0149	0.0102

From these data it appears that the process in question about doubles for each increase in temperature of 10°C., thus agreeing with a large number of chemical reactions. (**Van't Hoff, J. H.,** Lectures on theoretical and physical chemistry, translated by R. A. Lehfeldt. London, no date—author's preface dated 1898. Part I, p. 227 *et seq.*) See also: **Blackman, F. F.,** and **Matthaei, G. L. C.,** Experimental researches on vegetable assimilation and respiration. IV. A quantitative study of carbon-dioxide assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. London **B76**. 402–460. 1905. **Blackman, F. F.,** Optima and limiting factors. Ann. bot. **19**: 281–295. 1905. **Idem,** The metabolism of the plant considered as a catalytic reaction. Presidential Address, Bot. Sect. British Assoc., Dublin meeting, 1908. Also published in: Science, n.s. **28**: 628–636. 1908. Two criticial reviews of published data on photosynthesis may also be mentioned here; the first (**Brown, W. H.,** and **Heise, G. W.,** The application of photochemical temperature coefficients to the velocity of carbon dioxide assimilation. Philippine Jour. Sci. **12, C** (botany): 1–25. 1917.) interprets the data as indicating that temperature has little effect on the rate of the process, while the second (**Smith, A. M.,** The temperature coefficient of photosynthesis: a reply to criticism. Ann. bot. **33**: 517–536. 1919.) corroborates the interpretation that temperature has a pronounced effect on the rate.—*Ed.*

was able to show that when the stomatal pores are artificially plugged exchange of gases is retarded. A privet leaf (*Ligustrum vulgaris*), the upper surface of which was coated with petrolatum, decomposed 6.26 g. of carbon dioxide, but only 1.92 g. was decomposed by a similar leaf coated on the under surface. [Privet leaves have stomata only below, so that coating the upper surface did not close the pores.] Stahl[1] arrived at the same result. Parts of the lower surfaces of leaves that had been rendered free from starch were covered with a mixture of one part of beeswax and three parts of cocoa butter, and the leaves were then exposed to light; after being bleached with alcohol and then treated with iodine, the part that had been covered was brown, while the remainder of the leaf was dark blue (Fig. 18). Blackman's[2] results point to the same conclusion. The size of the stomatal openings is also important.[3]

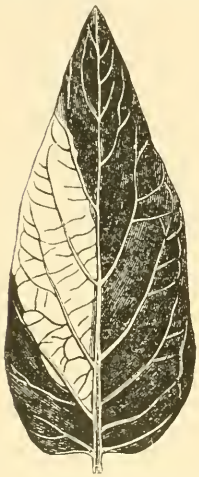


FIG. 18.—Privet leaf, the unshaded portion of which was covered with cocoa butter during exposure to light. This portion shows no starch reaction with iodine.

An adequate supply of water in the leaves is essential to the normal progress of photosynthesis; according to Sachs and Nagamatsz[4] no starch is formed by wilting leaves, a fact which Stahl believed to be due to the stomatal closure that accompanies wilting. This interpretation is supported by the observation that leaves in which the stomata remain open even in the wilted condition (*Rumex aquaticus*, *Caltha palustris*, *Hydrangea hortensis*, *Calla palustris*) still continue to accumulate starch after wilting has occurred.

Finally, an excess of salts in the soil has a retarding effect upon the rate of carbon dioxide decomposition. Schimper found that watering with sodium chloride solution caused development to cease in most plants (non-halophytes), through a checking of photosynthesis. According to Stahl this, also, is due to stomatal closure, caused by excess of salts. If the leaves are slightly wounded so as to facilitate entrance of carbon dioxide into the tissue, starch accumulates about the wound margins. True halophytes grow, though slowly, upon soils rich in salts, since their stomata do not close at all.

§9. Nutrition of Green Plants by Organic Compounds.—Green plants can also use as food organic compounds that are supplied from without.[5] This form of nutrition may go on simultaneously with the assimilation of carbon

[1] **Stahl, Ernst.**, Einige Versuche über Transpiration und Assimilation. Bot. Zeitg. 52^{I}: 117–146. 1894.

[2] **Blackman, F. Frost**, Experimental researches on vegetable assimilation and respiration.—No. I. On a new method for investigating the carbonic acid exchanges of plants. Phil. trans. Roy. Soc. London $B186^{I}$: 485–502. 1895. **Idem**, same title, No. II. On the paths of gaseous exchange between aerial leaves and the atmosphere. *Ibid.* **B**: 186^{I}: 503–562. 1895. See Sect. IV.

[3] **Kolkunov, V.**, Ueber die Abhängigkeit der Assimilation von der Grösse der Spaltöffnungen bei den Gramineen. [Abstract in German, pp. 381–382; text in Russian.] Jour. exp. Landw. 8: 369–382. 1907.

[4] **Nagamatsz, Atsusuke**, Beiträge zur Kenntnis der Chlorophyllfunktion. Arbeit. Bot. Inst. Würzburg 3: 389–407. 1888.

[5] Apparently carbon monoxide cannot be assimilated; see: **Kraschéninnikoff, Th.**, La plante verte assimile-t-elle l'oxyde de carbone? Rev. gén. bot. 21: 177–193. 1909.

dioxide from the air, which is especially true in the case of insectivorous plants.[1] These latter are green and can assimilate carbon dioxide, but, at the same time, they are provided with a characteristic mechanism for catching and digesting insects (Fig. 19). In this class, for instance, belongs the widely distributed sundew (*Drosera rotundifolia*), which grows in bogs. Its leaves are covered with pin-shaped tentacles or glands, which secrete a sticky fluid. As an insect alights upon the leaf, the tentacles bend toward it, a copious flow of an acid liquid

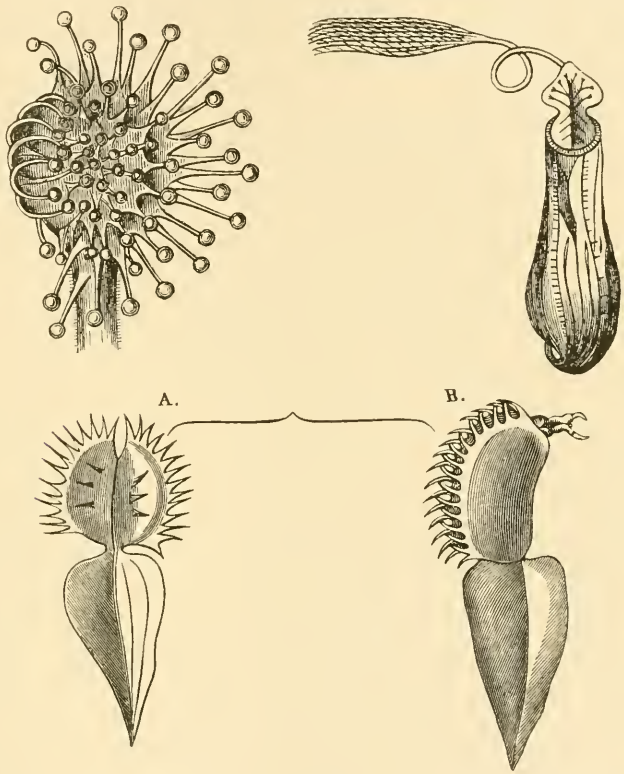


FIG. 19.—Above, a leaf of *Drosera rotundifolia*, whose tentacles on the left side have responded to a stimulus, and one of *Nepenthes gracilis*. Below, a leaf of *Dionaea muscipula*; *A*, open; *B*, closed, with an imprisoned earwig. (*After Pfeffer.*)

containing a pepsin-like enzyme takes place, and the insect is digested. Sundew can also digest and absorb lean meat and white of egg. In Nepenthes[2] a part of the petiole is modified into a tankard-shaped structure with the leaf-blade acting as a cover. The hollow portion contains a weakly acid solution, in which imprisoned insects are digested. Each leaf of *Dionaea muscipula* consists of a flattened petiole and a round leaf-blade divided by the midrib into halves, like the halves of an open mussel, separated by an angle of from 60 to

[1] **Darwin, Charles R.,** Insectivorous Plants. London, 1875.

[2] **Clautriau, G.,** La digestion dans les urnes de *Nepenthes*. Recueil Inst. Bot. Bruxelles **5**: 89–133. 1902. **Vines, S. H.,** The proteolytic enzyme of *Nepenthes* (III). Ann. bot. **15**: 563–573. 1901.

90 degrees. The free margin of each lobe is extended into sharp, slender teeth, and each lobe bears on its upper surface near the center three very elastic bristles. When an insect alights upon the leaf and touches a bristle, the valves quickly close together and a digestive fluid is secreted into the space between them.

If the ability to derive nutrition from complex organic compounds, independently of photosynthesis, is a special characteristic of the insectivores, nevertheless other plants that utilize the carbon dioxide of the air can also assimilate complex organic substances. Green water-plants thrive especially well in harbors where the water is very rich in organic compounds, in the neighborhood of canals and sewer outlets; for example, the algæ, *Ulva lactuca*, some species of the genera Bangia and Ceramium, and *Cystoseira barbata*. Also, some single-celled green algæ are known to grow excellently and retain their green color in pure culture in darkness, with organic substances supplied. Finally, it was proved by Böhm and other observers[1] that even green leaves that have been previously deprived of starch are able to assimilate various organic substances from solution and thus to form starch in darkness. In this manner starch can be formed from saccharose, glucose, fructose, lactose, glycerine, dextrine, mannite, melampyrite, and adonite.[2] Sapozhnikov[3] investigated this matter quantitatively. Leaves of *Astrapœa wallichii*, previously rendered starch-free, formed in seven days from 4.6 to 5.3 g. of starch, per square meter of leaf surface, when floating upon a 20-per cent. solution of cane sugar in darkness. Here assimilation is not limited for formation of starch, however; the amount of proteins also increases when leaves are grown upon cane-sugar solution in darkness, and respiration is accelerated. The ability to absorb organic compounds is even more pronounced in roots than in leaves. Many green plants possess mycorhiza (see Chapter IV) and grow on humus soils, and these probably assimilate organic materials. Light influences the absorption of organic compounds by green plants.[4]

According to the experiments of Reinhardt and Sushkov[5] the accumulation of starch in leaves floating upon cane-sugar solution depends upon a variety of conditions. This process occurs rapidly only at medium temperatures, while starch that was previously present disappears at higher or lower temperatures, in spite of the supply of sugar. Among poisons, some (quinin) hasten the first appearance of starch but prevent its continued accumulation; others (0.5 per cent. of caffein) favor the accumulation of starch.

[1] [**Boehm, Josef,** Ueber Stärkebildung aus Zucker. Bot. Zeitg. **41**: 33–38, 49–54. 1883. P. 35. **Idem,** Stärkebildung in den Blättern von Sedum spectabile Boreau. Bot. Centralbl. **37**: 193–201, 225–232. 1889. P. 200.] **Nadson, G.,** The formation of starch from organic substances by chlorophyll-bearing plant cells [Russian]. Trav. Soc. Imp. Nat. St-Pétersbourg **20**: (Sect. bot.): 73–122. 1889.

[2] **Treboux, O.,** Stärkebildung aus Adonit im Blatte von Adonis vernalis. Ber. Deutsch. Bot. Ges. **27**: 428–430. 1909.

[3] **Saposchnikoff, W.,** Ueber die Grenzen der Anhäufung der Kohlenhydrate in den Blättern der Weinrebe und anderer Pflanzen. (Vorläufige Mittheilung.) Ber. Deutsch. Bot. Ges. **9**: 293–300. 1891. P. 298. **Idem,** 1890, 1893. [See note 4, p. 31.]

[4] **Lubimenko, W.,** Influence de la lumière sur l'assimilation des matières organiques par les. plantes vertes. Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, **1**: 395–426. 1907.

[5] **Reinhard,** [**L. V.**] and **Suschkoff,** Beiträge zur Stärkebildung in der Pflanze. Beih. Bot. Centralbl. **18**: 133–146. 1904–1905.

Experiments in which green plants were supplied with organic nitrogenous compounds, in a chamber free from carbon dioxide, gave negative results.[1]

Summary

1. **Importance of Carbon Assimilation by Green Plants.**—Green plants form organic compounds from inorganic ones. Non-green plants and animals are unable to do this and are therefore all ultimately dependent on green plants for organic substances. . The study of plant physiology may begin by inquiring about photosynthesis of carbohydrates by the green parts of plants. These organic compounds are formed from carbon dioxide and water, by means of solar energy that is absorbed and transformed in the green tissues. Carbon dioxide is of course a carbon compound, but it is not combustible and is usually classed as inorganic. Combustible carbon compounds derived from organisms are capable of being burned in air because they are incompletely oxidized; when completing their oxidation these compounds absorb oxygen and produce carbon dioxide and water, and this process of combustion liberates energy (heat or light or both). A certain amount of sunlight energy is absorbed, and a corresponding amount of oxygen is eliminated, when carbon dioxide and water are combined by green plants, with the formation of carbon compounds.

2. **Exchange of Gases.**—Photosynthesis is accompanied by taking in of carbon dioxide and giving out of oxygen, as well as by absorption of solar energy, and the ratio of the amount of absorbed carbon dioxide to the amount of oxygen eliminated in the same period has been found to have a value somewhat less than unity. The process results in decomposition of carbon dioxide and water, and in the union of the carbon, the hydrogen, and some of the oxygen, to form carbohydrates; the rest of the oxygen is given off.

3. **Chlorophyll.**—The two green pigments that make it possible for carbohydrate photosynthesis to occur in green plant tissues when light is properly supplied are called chlorophyll, or, more correctly, the chlorophylls. Photosynthesis of carbohydrates from carbon dioxide and water does not occur in tissues that do not contain these pigments. The green pigments are named *chlorophyll a* and *chlorophyll b*. They occur in green leaves in about the proportions 72 to 28, by weight. Dissolved in ethyl alcohol, the first appears blue-green, the second yellow-green, by *transmitted* light. Both are fluorescent, the first appearing blood-red, the second brown-red, by *reflected* light. The two are alike in that each molecule contains 55 atoms of C, 4 atoms of N, and a single atom of Mg. The molecule of chlorophyll *a* contains 72 atoms of H and 5 atoms of O, while that of chlorophyll *b* contains 70 atoms of H and 6 atoms of O. Iron is necessary for the formation of the chlorophylls in plants, but does not occur in the pigments themselves.

The chlorophylls absorb light more or less completely according to the wave-lengths of the light that is supplied. Light of wave-lengths from about 640 to about 680 μ (red) is most completely absorbed. With wave-lengths shorter than about 475 μ (blue to ultra-violet) absorption is almost as complete. The spectrum of chlorophyll solution shows, between these two, several other ranges of wave-lengths, with less complete absorption, and very strong solutions show complete absorption throughout the entire range of visible light.—The chlorophylls are chemically some-

[1] **Grafe, Victor,** Untersuchungen über die Aufnahme von Stickstoffhaltigen organischen Substanzen durch die Wurzel von Phanerogamen bei Ausschluss der Kohlensäure. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien 118^{I}: 1135–1153. 1909.

what related to hemoglobin (which occurs in red blood-corpuscles of animals); they give several of the same decomposition products.

For the formation of chlorophyll in leaves, etc., the following conditions are essential: (1) light (within the limits of the visible spectrum and with different intensities for different kinds of plants); (2) temperature (from about 0°C. to about 45°C. as general limits; the range is usually narrower, differing for different kinds of plants); (3) iron (but the supply must be very small or poisoning results); (4) oxygen; (5) salts derived from the soil (containing K, Ca, Mg, N, P, S); (6) water-soluble carbohydrates.

4. **Pigments Accompanying Chlorophyll.**—Several other pigments accompany the chlorophylls, especially carotin and xanthophyll, which are generally present in cells with the green pigments, but often occur in the absence of the latter. Carotin is a hydrocarbon, with the formula $C_{40}H_{56}$. It forms crystals that appear blue-green by reflected light and orange-red by transmitted light. It is insoluble in water, readily soluble in ether, carbon bisulphide, etc., and is readily oxidized. In leaves it varies in amount, according to the light intensity, temperature, etc. It occurs in all parts of plants.—Xanthophyll resembles carotin but contains some oxygen; it has the formula $_{40}CH_{56}O_2$.

5. **Influence of Light in Carbohydrate Photosynthesis.**—Light impinging on leaves is partly reflected, partly absorbed, and partly transmitted. Only that which is absorbed can influence chemical processes within the leaves. The absorbed portion may have various qualities (according to the proportions of the different ranges of wave-length that are present) and various total intensities. The range of wave-lengths approximately corresponding to our visual range of red and orange appears usually to be most effective in furnishing energy for photosynthesis, but the rest of the visible range of wave-lengths is not without effect. The proportional distribution of total light energy among the several ranges of wave length varies greatly in nature. When the relations between light quality and carbohydrate photosynthesis are to be dealt with, it is necessary to consider the energy-supplying power of any wave-length range of absorbed light. It has been suggested that the rate of the process may be proportional to the energy value of the absorbed light, other conditions being adequate and constant throughout the series of comparisons. The absorbing power of chlorophyll-bearing tissue, for the different ranges of wave-lengths, is greatly influenced by the amount of chlorophyll present and by the presence of pigments other than chlorophyll—also by the cell structures of the tissues.—Considering simply the total intensity of sunlight, carbohydrate photosynthesis proceeds with intensities between a minimum and a maximum, with an optimum intensity somewhere within the range. Shade-plants (as beech) have a low range of intensities for the process, while sun-plants (as pine) have a high range. Cave mosses thrive with very weak illumination.

6. **Products of Carbohydrate Photosynthesis.**—If a living green plant that forms starch be kept in darkness till all starch has disappeared from the chlorophyll-bearing cells, and if it be then exposed to suitable light, starch grains soon appear in the cells. But starch is not the first product of the photosynthetic process, for starch is formed from a water-soluble sugar (such as dextrose), not directly from carbon dioxide and water. There are plants that do not form starch, and these show an increased amount of sugar when they are brought into light after a prolonged period in darkness. A supply of carbon dioxide is of course necessary, in the surrounding air, and the formation of sugar or starch proceeds parallel to the absorption of carbon dioxide by the plant in this kind of a test. Of course the active cells are plentifully supplied with water, which is the other necessary material. Besides sugar, a prominent product of

this process is oxygen, most of which escapes from the green tissues into the surroundings.

6a. Chemistry of Carbohydrate Photosynthesis.—Baeyer's hypothesis supposes that carbon dioxide and water are decomposed, that some free oxygen is produced, and that the remaining carbon, hydrogen, and oxygen are combined to form formaldehyde (CH_2O), the latter being polymerized, with the formation of dextrose ($C_6H_{12}O_6$). The hypothesis is represented by the equations: (1) $CO_2 + H_2O = CH_2O + O_2$ and (2) $6CH_2O = C_6H_{12}O_6$. Traces of formladehyde have been found in green tissues, and green plants in light have been experimentally shown to be able to increase their carbohydrate content when supplied with this substance as the only source of carbon. But formaldehyde is a violent poison and can never accumulate considerably in living tissues. It is supposed that this substance generally polymerizes as rapidly as it is formed. If the hypothesis is true, light appears necessary for the polymerization of formaldehyde, as well as for its formation and for the antecedent decomposition of carbon dioxide and water. Many other hypotheses have been suggested, and the chemistry of this photosynthetic process is still to be worked out.—More than 90 per cent. of the dry weight of the plant is derived from the carbon dioxide and water used in the process here considered; the rest is derived from mineral salts absorbed from the soil solution.

7. Assimilation of Solar Radiant Energy by Green Plants.—The formation of carbohydrates in green plants necessarily results in the storage of potential energy, in an amount equivalent to the energy that would be freed by the complete oxidation or burning of the carbohydrates formed. The fuel values of wood and coal are proportional to the potential energy stored in these substances and set free when they are burned. This energy is a part of that which was absorbed from sunlight when the plants from which these fuels have been derived were growing. The stored solar energy of coal has lain dormant for ages, that of wood generally for years. Calculations indicate that 2.2–3.6 gram-calories of energy is stored for each gram of carbon dioxide decomposed in photosynthesis. Experiments have shown, however, that plants accumulate, as potential energy in their carbon compounds, less than 0.5 per cent. of the radiant energy that reaches them as sunlight.

8. Influence of Conditions on Carbohydrate Photosynthesis.—*Internal* conditions influencing the rate of carbohydrate photosynthesis are: (*a*) the amount of chlorophyll present; (*b*) anatomical and histological structure, especially arrangement and size of stomata; (*c*) condition of stomata—whether open, closed, partly closed, etc.; (*d*) turgor condition—whether the leaf is wilted, etc. (this is perhaps covered by *c*); (*e*) the rate at which products of the process leave the leaf; (*f*) the ability of the leaf to absorb light (may be included under *b*); (*g*) leaf temperature.

External conditions influencing the rate of this process are: (*a*) the rate of supply of carbon dioxide; (*b*) the quality—wave-lengths—of the light received; (*c*) the rate of light-energy absorption—intensity of each group of wave-lengths and time during which leaf is exposed to them; (*d*) the temperature of the surroundings—which mainly controls leaf temperature; (*e*) other external conditions whose influence is not yet so well understood.

9. Nutrition of Green Plants by Organic Compounds.—Some green plants (as, for example, the insectivorous forms, Drosera, Nepenthes, Dionæa, etc.) are able to absorb considerable amounts of ready-made carbohydrates, etc., from the surroundings. Many other green plants have this ability to a smaller degree. Of course the non-chlorophyll-bearing parts of green plants, regularly, derive their carbohydrates from the tissues that bear chlorophyll.

CHAPTER II

ASSIMILATION OF CARBON AND OF ENERGY BY PLANTS WITHOUT CHLOROPHYLL

§1. **General Discussion.**—Most plants that are without chlorophyll and are, in consequence, unable to assimilate the energy of sunlight, do not have the power to transform non-combustible inorganic substances into organic compounds. As will appear later, in order to form their various organic substances, green plants require (besides carbon dioxide from the air and water from the soil) nitrogen, potassium, calcium, magnesium, iron, sulphur and phosphorus, all of which occur in the form of various salts in the soil. From the preceding discussion of chlorophyll (see Chapter I) it appears that no plant without chlorophyll can utilize the energy of sunlight to manufacture combustible organic matter out of such substances. Most non-green plants must use, as sources of both energy and material, organic compounds that have already been formed; they are thus more nearly related to animals than to green plants, as far as their nutrition is concerned. But organic compounds are not the only substances that can be oxidized. This property belongs also to various inorganic substances, such as ammonia, hydrogen sulphide and hydrogen, which thus contain stored energy. As we have previously seen (page xxviii), the heat of combustion of ammonia is greater than that of starch. The researches of recent years have shown that such substances can serve as sources of nutrition for certain plants without chlorophyll. On the basis of their mode of nutrition, plants without chlorophyll may be divided into two groups: (1) plants that derive their energy from organic compounds, and (2) plants that derive it from inorganic substances.

§2. **Assimilation of Energy from Organic Compounds by Plants without Chlorophyll.**—Most bacteria, yeasts, fungi and the non-green seed-plants obtain their nutrition from previously formed organic compounds. To study the nutritional requirements of these forms, culture media containing various nutritive substances are employed. It was formerly thought that the same nutrient medium should be suitable for all the simpler non-green forms, but this is not so. In higher plants, specialization—*i.e.*, adaptation to surrounding conditions—is accompanied by peculiarities of external form as well as of anatomical structure. On the other hand, the lower plants, such as bacteria and yeasts, are marked by their structural similarity and simplicity. It was supposed, therefore, that such similarity of structure was accompanied by a similarity in the characteristic life processes, and this, in turn, led to the supposition that the nutritive processes must be more or less uniform in these lower forms. The most recent investigations have shown, however, that, in spite of the simple structure of microörganisms

(more properly, just because of this very simplicity) they usually exhibit far-reaching physiological peculiarities. Each one of these organisms carries out its own little work, but it constitutes a very important link in the processes of nature. For example, the presence of two kinds of bacteria appears to be requisite for the oxidation into nitric acid of the ammonia present in the soil. One of these (Nitrosomonas) carries the oxidation as far as nitrous acid, the other (Nitrobacter) oxidizes this to nitric acid. Ammonia is essential as nutrient material for the first form and nitrous acid is a waste. But this by-product constitutes an essential food substance for the other form. Is it possible, then, to conceive of some nutrient medium that would be equally well suited for the nutrition of both these bacteria? This question must receive a negative answer; a medium must be used that is favorable only to the microörganism under investigation, and that is especially adapted to its particular requirements. The use of such media is highly important if pure cultures are desired. This use has been designated by Vinogradskii as the method of "selective culture." A culture is selective if it promotes only a certain function, or if it promotes a function which is as restricted as possible. The more closely limited or exclusive are the conditions, the more favorable will these conditions be for one species possessing a particular property or function, in contrast to others not so endowed, and the growth of these latter in a medium thus alien to them will be quite impossible or at least very difficult. In thus assisting the desired microörganisms in their struggle for existence, we increase their numbers in our cultures and thereby render their discovery easier. When a specific bacterium has once been found, it is thus usually possible to discover also the method by which it may be isolated in pure culture. On this general principle is based the now frequent employment of many different kinds of nutrient substrata, both liquid and solid. The first attempt to prepare an artificial nutrient medium for microörganisms, was made by Pasteur,[1] whose solution for the culture of yeast had the following composition: water, 100 g.; ammonium tartrate, 1 g.; saccharose, 10 g.; and yeast ash, 0.075 g.

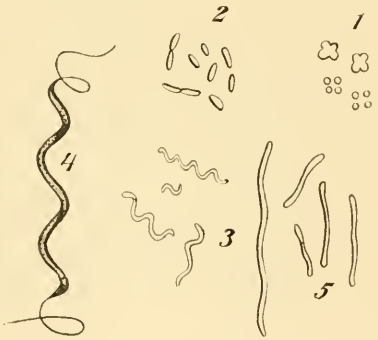


FIG. 20.—Various forms of bacteria.

Meat extract is used most commonly for the culture of bacteria (Fig. 20). The addition of gelatine to peptone bouillon (10 per cent. of gelatine in winter and 15 per cent. in summer) produces a solid substratum. Agar-agar may be used instead of gelatine. Besides the various kinds of meat extracts, milk, blood serum, yeast water, beer-wort and other similar materials may be used. Among other things, cylinders cut from potato tubers may be employed as solid media.

[1] **Pasteur, Louis,** Mémoire sur la fermentation alcoolique. Ann. chim. et phys. *III*, **58**: 323–426. 1860.

Beer-wort is the best nutrient medium for the culture of yeast.[1] Other liquids are used, however, among which may be mentioned Pasteur's solution as given above, grape juice, the juice of various other fruits and berries, and other materials containing sugar. Hansen has carried out very exhaustive studies upon yeasts and has established, among others, the following important species.[2]

Saccharomyces cerevisiæ I. Hansen. An English top-fermentation yeast, which produces, in beer-wort at room temperature, from 4 to 6 per cent. of alcohol. In the resting condition the plant consists of single cells, which begin to multiply by budding when placed in beer-wort. The young generation consists of large spherical or oval cells (Fig. 21). After the temination of the primary fermentation a scum appears on the surface of the fermenting liquid and on this a continuous membrane of yeast-cells is formed. The general appearance of these cells is different from that of the sedimentary forms; much elongated cells are found here (Fig. 22). In the surface membrane of old cultures occur very much elongated cells that are entirely unlike the young sediment cells from which they have developed (Fig. 23). This film formation

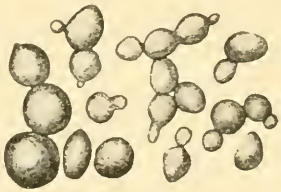


FIG. 21.—*Saccharomyces cerevisiæ I.* Young cells from the sediment of the beer-vat. (*After E. Hansen.*)

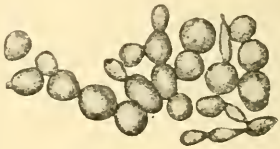


FIG. 22.—*Saccharomyces cerevisiæ I.* Surface film at 15–16°C. (*After E. Hansen.*)

furnishes a striking example of the great variability in form, that is characteristic of yeast cells.

In order to obtain ascospores young cultures must be used, and it is also essential that air be plentifully supplied. Little plaster of Paris disks prepared with special moulds are used for this purpose. These are placed in small, shallow glass pans (Petri dishes), covered with similar pans of slightly greater diameter, and then sterilized. A few drops from a day-old culture of yeast cells are placed upon one of these plaster disks. Sterilized water is poured into a dish around the disk, to keep the latter constantly moist. After some time the ascospores are formed. Temperature exerts a pronounced influence upon their formation. With the same temperature, ascospores of different species develop at different rates, and this fact is made use of in indenti-

[1] **Jörgensen, Alfred P. C.,** Die Mikroörganismen der Gärungsindustrie. 4te Aufl. Berlin, 1898. **Idem,** Microörganisms and fermentation. Philadelphia, 1911. **Lindner, Paul,** Mikroskopische Betriebskontrolle in den Gärungsgewerben. 2te Aufl., Berlin, 1898. (5te Aufl., Berlin, 1909.) [**Hansen, Emil Chr.,** Practical studies in fermentation. Transl. by Alex. K. Miller. 227 p. London and New York, 1896.—See also the references on brewing, etc., given on p. 181.]

The Carlsberg Laboratory in Copenhagen is especially interested in the study of fermentation organisms. It publishes a journal devoted to this study, entitled "Meddeledser fra Carlsberg Laboratoriet."

[2] More information upon top and bottom fermentation will be found in Chapter VIII of this Part.

FIG. 23.—*Saccharomyces cerevisiæ I.* Film of an old culture. (*After E. Hansen.*)

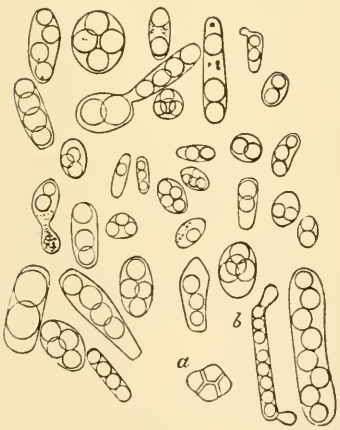


FIG. 24.—*Saccharomyces pastorianus I.* Ascospores. (*After E. Hansen.*)

FIG. 25.—*Saccharomyces pastorianus III.* Young cells of the sediment. (*After E. Hansen.*)

fying the different yeasts, particularly in technical analysis for distinguishing wild from cultivated forms.

Saccharomyces pastorianus I. Hansen (Fig. 24). This is a bottom-fermentation yeast and consists mainly of elongated cells, but round and oval cells also occur. This yeast is frequently present in the air in breweries. It imparts to the beer a disagreeable, bitter taste and an unpleasant odor.

Saccharomyces pastorianus III. Hansen. This top-fermentation yeast produces a turbid condition in beer (Fig. 25).

Saccharomyces anomalus Hansen. This species is distinguished by its characteristic ascospores, which have the form of hemispheres, with projecting rims at their bases (Fig. 26).

Besides the species mentioned here, which are among those thoroughly investigated by Hansen, a great many other yeasts, both wild and cultivated, are known. Some of the cultivated varieties are employed in the brewing industry, some in distilleries, some in the manufacture of berry or fruit wines, and still others in the preparation of compressed yeast for bakers' use.

The moulds (Fig. 27) are not very exacting as to their nutrition,

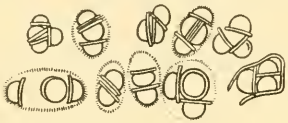


FIG. 26.—*Saccharomyces anomalus.* Ascospores.

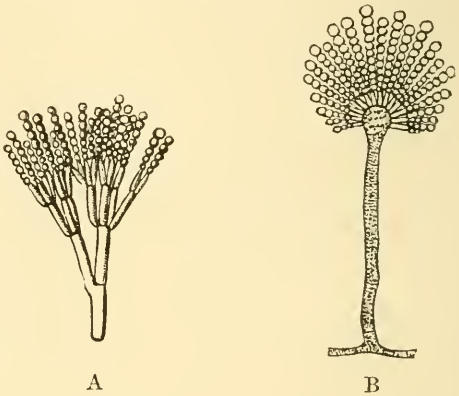


FIG. 27.—*A, Penicillium glaucum; B, Aspergillus glaucus.* A conidiophore, in each case.

for they can grow upon a very great variety of materials. Among artificial liquid media for mould culture, Raulin's[1] solution is the best known; its formula follows:

Water	1500.0 g.
Saccharose	70.0 g.
Tartaric acid	4.0 g.
Ammonium nitrate	4.0 g.
Ammonium phosphate	0.6 g.
Ammonium sulphate	0.25 g.
Potassium silicate	0.07 g.
Potassium carbonate	0.6 g.
Magnesium carbonate	0.4 g.
Zinc sulphate	0.07 g.
Ferric sulphate	0.07 g.

Fermentation phenomena often accompany the nutrition of the moulds and bacteria. There is still very little known concerning the nutrition of the higher fungi.

[1] **Raulin, Jules,** Etudes chimiques sur la végétation. Ann. sci. nat. Bot. *V*, **11**: 93–299. 1869.

Almost the only definitely known fact concerning the nutrition of seed-plants without chlorophyll is that some are saprophytes and others parasites. The former utilize decomposition products from plants and animals, while the latter attach themselves to living plants and derive nourishment therefrom.

The widely distributed dodder (species of Cuscuta) is an example of a parasite. It is parasitic upon nettles, hops and many other plants (Fig. 28). Parasitism exhibits such a high state of development in some flowering plants without chlorophyll that they possess neither root nor stem, nor have they any leaves. The entire plant body here resembles a fungus in its structure, consisting of branching filaments each composed of a row of cells, very similar to fungus hyphæ. The Balanophoreæ, Hydnoreæ and Rafflesiaceæ, are examples of such plants. The hypha-like body of these plants develops within various trees and derives nourishment therefrom after the manner of

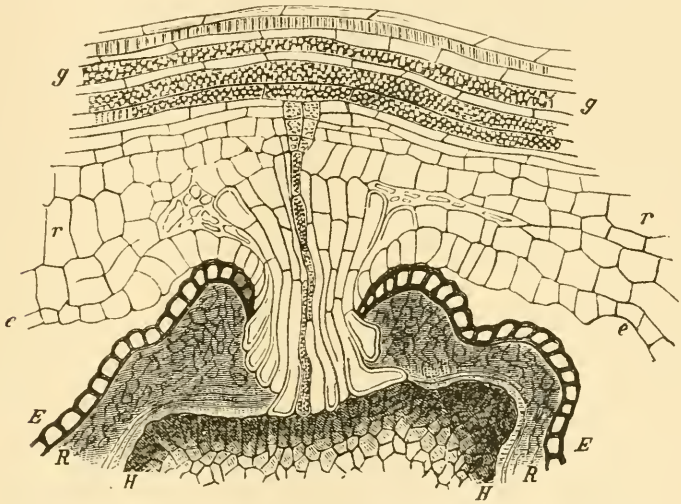


FIG. 28.—Section of stem of *Cuscuta europæa*, attached, by means of its haustorium, to the stem of a nettle. *E* represents the epidermis of the nettle.

many fungi. The flower buds and flowers of these non-green parasites appear upon the branches of the host only during the flowering season of the latter. It then appears, at first glance, as though the plant infested by the parasite were bearing two kinds of flowers. In reality, however, some of these are the true flowers of the host plant, while the others belong to the parasite. Fig. 29 shows a portion of an underground stem of a host plant, bearing its own flower buds and a mature flower of a parasite, *Hydnora africana*.

§3. Assimilation of Energy from Inorganic Substances by Plants without Chlorophyll.—Some bacteria are so constituted as to be able to obtain their energy from oxidizable inorganic substances that are common on the earth. Of these the nitrifying bacteria, which oxidize ammonia into nitric acid, are the most important. The absence of organic substances is necessary for their successful growth. Vinogradskii succeeded in obtaining a pure culture of

nitrifying bacteria only, by preparing a nutrient solution containing no organic substances. This nutrient medium[1] contained 1 g. of ammonium sulphate and 1 g. of potassium phosphate, dissolved in a liter of water. From 0.5 to 1.0 g. of basic magnesium carbonate was added to each 100 cc. of this solution. Nitrifying bacteria were able to develop excellently in this medium; they oxidized ammonia to nitric acid and formed an appreciable quantity of organic substance, thus assimilating the carbon dioxide of the air without the agency of sunlight. Bacteria that need organic substances for their nutrition could not develop in such a medium.

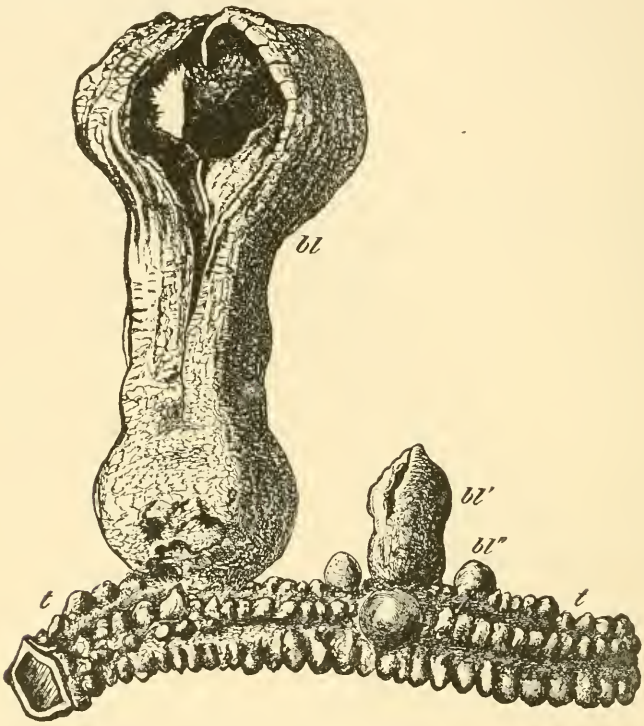


FIG. 29.—*Hydnora africana.* *t*, part of the underground stem of the host plant; *bl*, one of the mature flowers; *bl'*, *bl''*, flower buds of the parasite. (⅔ natural size.) (*After Sachs.*)

Without the agency of sunlight as source of energy, green plants are unable to produce organic substance from the inorganic materials that serve as nutrients for these forms. As has been said, there are other inorganic substances, however (such as ammonia and hydrogen sulphide) that can serve as sources of energy for such plants as the bacteria just mentioned. These substances are common in nature, being frequently of organic origin as decomposition products of complex organic compounds, and, although they do not contain carbon (which is present in all organic compounds), yet they do possess the power

[1] [**Winogradsky, S.**, Recherches sur les organismes de la nitrification. I. Ann. Inst. Pasteur 4: 213–231 1890. **Idem**, same title, II. *Ibid.* 4: 257–275. 1890. **Idem**, same title, III. *Ibid.* 4: 760–771. 1890. **Idem**, same title, IV. *Ibid.* 5: 52–100. 1891. [**Idem**, same title, V. *Ibid.* 5: 577–616. 1891. See No. IV, especially.]

to burn readily; *i.e.*, to liberate heat. On this account these oxidizable inorganic substances can supply energy for these bacteria. Thus, nitrifying bacteria utilize ammonia, and sulphur bacteria make use of hydrogen sulphide.

To obtain a solid substratum for cultures where organic substances must be avoided, silicic acid[1] may be used instead of gelatine or agar-agar.

Vinogradskii[2] also proved that bacteria living in sulphur springs, as Beggiatoa and some other species, use hydrogen sulphide as a source of energy. This is first oxidized only to sulphur and water; $H_2S + O = H_2O + S$. The sulphur thus formed accumulates within the cells, to be further oxidized, in the presence of carbonates (*e.g.*, calcium carbonate), to form calcium sulphate and carbonic acid. The sulphur bacteria play a very important rôle in the economy of nature; without them the circulation of sulphur might be impossible.

In order to obtain sulphur bacteria, freshly cut pieces of roots of *Butomus umbellatus*, with the mud clinging to them, are placed in a deep vessel, in from 3 to 5 l. of water; some calcium sulphate is added and the vessel is left uncovered at room temperature. After several days the formation of hydrogen sulphide is evident, consequent upon the decomposition of calcium sulphate by various bacteria contained in the mud. Some time after the appearance of hydrogen sulphide the development of sulphur bacteria begins. They usually collect at some distance from the free surface of the liquid and, as they move upwards and downwards, they sometimes absorb hydrogen sulphide and sometimes oxygen.

When grown upon a microscope slide, in a liquid containing hydrogen sulphide, the sulphur bacteria assemble to form a ring, about a millimeter from the edge of the cover glass. If the drop of liquid is not covered they do not develop at all. There is therefore a definite optimum of oxygen supply for these bacteria. According to the researches of Yegunov,[3] this point is well brought out by growing them in deep vessels. A bacterial membrane is formed at a certain distance from the surface of the liquid and short, tassel-like outgrowths project downwards from this membrane. A part of such a membrane with its projections is shown, enlarged, in Fig. 30. If these outgrowths are examined with a horizontal microscope it becomes evident that they consist of bacterial cells that are moving up and down with a boiling motion, like water in a spring.

The occurrence of hydrogen sulphide is not confined to bogs and sulphur springs, for this substance is also found in the sea. The water of the Black Sea below a depth of about 200 m. becomes richer in hydrogen sulphide as the depth increases. One hundred liters of water, collected at the depths given, contained the following amounts of hydrogen sulphide.

[1] **Omeliansky, V.,** Sur la culture des microbes mitrificateurs du sol. Arch. sci. biol. St.-Pétersbourg 7: 291–302. 1899.

[2] **Winogradsky, Sergius,** Ueber Schwefelbacterien. Bot. Zeitg. 45: 489–507, 513–523, 529–539, 545–559, 569–576, 585–594, 606–610. 1887. **Nathansohn, Alexander,** Ueber eine neue Gruppe von Schwefelbacterien und ihren Stoffwechsel. Mittheil. Zool. Sta. Neapel 15: 655–680. 1902. **Beijerinck, M. W.,** Ueber die Baketerien welche sich im Dunkeln mit Kohlensaüre als Kohlenstoffquelle ernähren können. Centralbl. Bakt. *II*, 11: 493–599. 1904. **Omelianski, W.,** Ueber eine neue Art farbloser Thiospirillen. *Ibid. II*, 14: 769–772. 1905.

[3] **Yégounow, M.,** Sur les sulfobactéries des limans d'Odessa. Arch. sci. biol. St.-Pétersbourg 3: 381–397. 1895. **Idem,** Die Mechanik und Typen der Teilung der Bakterienscharen. Centralbl. Bakt. *II*, 4: 97–109. 1898.

Depth in the Black Sea, *meters*	H_2S Content per 100 L. *cc.*
215	33
432	222
2040	555
2525	655

In the mud of the sea-bottom are therefore going on various kinds of fermentation, which are accompanied by the elimination of hydrogen sulphide.[a] Only because of the presence of sulphur bacteria is the hydrogen sulphide prevented from reaching the upper layers of water.

Nitrifying and sulphur bacteria use ammonia and hydrogen sulphide, which are injurious to other organisms, and aid in preventing the accumulation of these substances upon the surface of the earth; oxidizing them to nitric and sulphuric

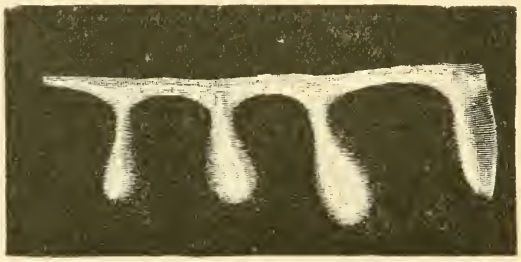


Fig. 30.—Part of a membrane of sulphur bacteria, magnified 11 times. (*After Yegunow.*)

acids, they bring these substances again into the general circulation of materials in nature.

Besides ammonia and hydrogen sulphide, hydrogen is also produced in large amounts by the decomposition of complex organic compounds, and yet it is present only in minimal quantities in the atmosphere. According to various determinations, the amount of hydrogen in the air varies between 0.0003 and 0.01 per cent. It therefore appears that processes must occur on the earth, by which hydrogen is combined and so started anew in the general circulation of materials.

The researches of Kaserer[1] have shown that there are special bacteria that utilize hydrogen. Viewed from the standpoint of thermo-chemistry, hydrogen represents the best nutrient substance. Its heat of combustion is eight times that of starch; a gram of starch gives out during combustion but 4.0 kg.-cal., of heat, while a gram of hydrogen gives out 34.6 kg.-cal. (see page xxviii). Certain soil bacteria, such as *Bacillus pantotrophus* and *Bacillus oligocarbophilus*,

[1] **Kaserer, Hermann,** Die Oxydation des Wasserstoffes durch Mikroörganismen. Centralbl. Bakt. *II* 16: 681–696, 769–775. 1906. **Lebedeff, A. F.,** Ueber die Assimilation des Kohlenstoffes bei wasserstoffoxydierenden Bakterien. Ber. Deutsch. Bot. Ges. 27: 598–602. 1909. **Nabokich, A. J.,** and **Lebedeff, A. F.,** Ueber die Oxydation des Wasserstoffes durch Bakterien. Centralbl. Bakt. *II*, 17: 350–355. 1907.

[a] This deduction is of course not strictly accurate; although perhaps most of the hydrogen sulphide, ammonia and hydrogen in nature is of organic origin, these substances are also produced, to some extent at least, quite independently of organisms.—*Ed.*

utilize hydrogen.[1] The former can derive its nourishment from organic compounds but it can also grow in purely inorganic media, in which case it assimilates carbon dioxide and hydrogen from the atmosphere and forms formaldehyde according to the equation, $H_2CO_3 + 2H_2 = CH_2O + 2H_2O$. Niklevskii[2] has isolated two bacteria (*Hydrogenomonas nitrea* and *H. flava*) that can live upon an inorganic substratum with an atmosphere of hydrogen and oxygen containing some carbon dioxide. They form organic compounds from hydrogen and carbon dioxide, which are then oxidized to carbon dioxide and water during respiration. The assimilation of hydrogen ceases when they are grown upon organic substances.

In all cases here described, of nutrition of bacteria by inorganic substances, the production of organic compounds occurs without the agency of sunlight. The formation of hydrogen, hydrogen sulphide and ammonia (by reduction of oxidized compounds existing in nature, such as water, sulphuric acid and nitric acid), goes on at the expense of radiant energy assimilated in green leaves, however. Therefore it is indirectly at the expense of this energy that nitrifying bacteria, sulphur bacteria and hydrogen bacteria are able to exist.[b]

[1] Methane (CH_4), which is frequently given off during the putrefaction of organic substances, can also serve as a nutrient material for some bacteria. [See: **Söhngen, N. L.,** Ueber Bakterien, welche Methan als Kohlenstoffnahrung und Energiequelle gebrauchen. Centralbl. Bakt. II, **15**: 513–517. 1906.]

[2] **Niklewski, Bronislaw,** Ueber die Wasserstoffoxydation durch Mikroörganismen. Jahrb. wiss. Bot. **47**: 113–142. 1910.

[b] In the foregoing discussion the terms "combustible" or "oxidizable" and "non-combustible" or "non-oxidizable" substances should be considered as synonymous with the more accurate ones "substances of high energy content" and "substances of low energy content." Although plant physiology has never yet received adequate treatment from the standpoint of energy transformations, some of the more general principles of such a treatment are well recognized and are pertinent in the present connection. Energy can no more be destroyed or created than can matter, so that when compounds of high energy content (carbohydrates, proteins, etc.) are formed from compounds of lower energy content (carbon dioxide, water, inorganic salts, etc.) energy must be supplied from some source other than the reacting substances themselves. Since the reverse process *yields* energy it is conceivable that some of the energy obtained by the oxidation of large organic molecules may enter into reaction by which other complex compounds may be formed. This appears to take place to some extent in green plants, in the formation of proteins, cellulose, etc., and in parasites and saprophytes. It is also conceivable that other substances that yield energy upon oxidation may enter into analogous reactions. That this possibility is realized in the cases of some bacteria seems to be true, and is one of the chief contributions that the investigation of these forms has made to general physiology. Beggiatoa, which the author mentions, appears to be able to form complex organic molecules from carbonates by means of the energy derived from the oxidation of hydrogen sulphide. (See: **Keil, Friedrich,** Beiträge zur Physiologie der farblosen Schwefelbakterien. Cohn's Beiträge zur Biol. d. Pflanzen **2**: 335–372. 1912.)

Bacteria that produce hydrogen sulphide must derive the necessary energy from other reactions that *yield* energy, as from the oxidation of carbohydrates. Many other colorless bacteria are similar in this respect. Besides the authors already cited in the text, see: **Keil,** 1912 (just cited). **Hinze, G.,** Thiophysa volutans, ein neues Schwefelbakterium. Ber. Deutsch. Bot., Ges. **21**: 309–316. 1903. **Molisch, Hans,** Neue farblose Schwefelbakterien. Centralbl. Bakt. *II.* **33**: 55–62. 1912. **Lauterborn, Robert,** Eine neue Gattung der Schwefelbakterien (Thyoploca schmidlei, *nov. gen., nov. spec.*). Ber. Deutsch. Bot. Ges. **25**: 238–242. 1907.

Other bacteria oxidize sulphites, the liberated energy apparently enabling them to form

§4. **Distribution of Microörganisms in Nature.**—The study of microörganisms is possible only with the aid of the microscope, and their discovery was impossible until magnifying glasses became available. The Columbus who discovered the world of the lowest organisms, which are ordinarily invisible, was a Dutch lens-maker of Delft, Anton van Leeuwenhoek. He succeeded in making magnifying glasses that magnified 100 and even 150 diameters. When, in 1675, he examined a drop of rain water that had stood for several days in a barrel, using one of his glasses, he observed a vast number of extremely small organisms moving hither and thither in the water. The number of these organisms approached 10,000 in a single drop. No such organisms were to be seen in freshly collected rain water, and Leeuwenhoek therefore concluded that the germs of these must have fallen into the water from the air.

The question then arose as to the origin of these extremely small organisms, and this became the subject of a very lively polemic. It is well known that infusions of most organic materials, such as meat and vegetable matter, decompose very easily. Microscopical examination of material undergoing decomposition always shows the presence of microörganisms. The promptness with which they appear led to the conclusion that we have here a spontaneous generation (*generatio spontenea*) of the lowest forms of life out of various organic substances.

The theory of spontaneous generation has had many adherents, even until recent times. Thus, van Helmont (1577–1644) was the author of a recipe for the production of mice from meal. It was maintained that maggots (fly larvæ) arise by spontaneous generation in meat. Even after it had been provided by exact experimentation that neither mice nor maggots can be produced *de novo*, and that such forms must arise by propagation, still the conviction persisted for a long time that the tiny, microscopic organisms may develop by spontaneous generation. As early as 1776 Spallanzani proved experimentally that this theory was incorrect. He showed that no animalcules appeared in an hermetically sealed vessel containing an infusion of organic material, no matter how long this was allowed to stand, provided the infusion had been first boiled for three-quarters of an hour. After such a vessel had been opened, however, the contents soon began to putrefy; because germs entered from the air, as Spallanzani maintained. Although the adherents of the theory of spontaneous

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complex carbon compounds from mineral carbonates and bicarbonates. (See **Nathansohn,** 1902, and **Beijerinck,** 1904. [Note 2, p. 49.]) In addition to these there are still others that oxidize ferrous compounds to the ferric form.[1] See: **Winogradsky, S.,** Ueber Eisenbakterien. Bot. Zeitg. **46**: 261–270. 1888. **Molisch, Hans.** Die Eisenbakterien. Jena, 1910. **Lieske, Rudolf,** Beiträge zur Kenntnis der Physiologie von Spirophyllum ferrugineum Ellis, einen typischen Eisenbakterium. Jahrb. wiss. Bot. **49**: 91–127. 1911. **Idem,** Untersuchungen über die Physiologie eisenspeichernder Hyphomyceten. *Ibid.* **50**: 328–354. 1911.

Since the forms, or kinds, of energy are mutually transformable it is possible that energy for the syntheses that occur in organisms may be derived not only from chemical reactions and light but also from other immediate sources, such as the radiant energy of heat and electricity. The heat of the medium in which the reactions occur is of course a very important source of energy, not generally discussed in this connection.—*Ed.*

generation were not convinced by the experiment of Spallanzani, nevertheless it received a practical application at the hands of a French cook, Francois Appert, who started a factory for making preserves. He found that it was possible to keep meats, vegetables and liquids unspoiled for unlimited periods of time, if these materials were placed in hermetically sealed jars and then heated in boiling water. Appert published his experiments in a book which passed through many editions;[1] the book brought him fame, the preserves brought him a fortune. We have here a conspicuous example of the dependence of technical arts upon theoretical knowledge; Spallanzani, in solving the purely philosophical question of the origin of living things on the earth, thereby gave Appert the opportunity to found a new industry.

Since the objection was raised against Spallanzani's experiment, that the closed vessels contained an inadequate supply of air and that the quality of what air there was must be greatly impaired by the high temperature, Franz Schultze performed the following experiment in 1836. A glass flask (Fig. 31) half full

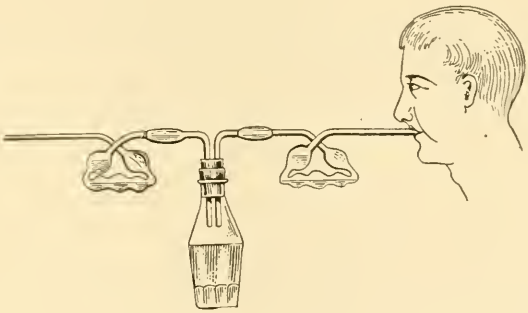


FIG. 31.—Arrangement of bottle and potash bulbs in Schultze's experiment.

of an organic infusion and tightly closed with a cork stopper, through which two bent glass tubes were passed, was subjected to active boiling for some time. While hot steam was still escaping from both tubes he attached a potash bulb to each, one filled with potassium hydroxide solution and the other with sulphuric acid, after which the apparatus was allowed to cool. Twice a day, for three months thereafter, air was drawn through the flask, entering through the sulphuric acid and passing out through the alkali. No organisms of any kind were found in the solution. All the germs present in the entering air were removed by the sulphuric acid. In this experiment the air retained its usual composition and was not heated.

But this experiment did not seem to be entirely convincing, and it was only by the remarkable investigations of Pasteur that the question of spontaneous generation was finally and conclusively settled in the negative. Pas-

[1] [**Appert, Charles,** L'art de conserver pendant plusieurs années toutes les substances animales et végétales. 2nd ed. Paris, 1811. **Idem,** Le livre de tout les ménages ou l'art de conserver pendant plusieurs anées les substances animales et végétales. 3rd ed. Paris, 1813. A 5th ed. was published in 1842, or earlier. None of these has been seen in preparing this note; the references are taken from: Catalogue général des livres imprimés de la Bibliothèque Nationale, Paris 3: 736. 1899.—*Ed.*

teur (1857) closed glass flasks of various solutions with cotton plugs and subjected them to prolonged boiling. If the boiling had been continued sufficiently long the solution in the flasks remained unchanged and free from microörganisms for an indefinite period of time. The air that entered the flasks during cooling was filtered through the cotton plugs, in which all the germs that it originally held were left behind. Since the spores of some bacteria withstand a single, though long-continued boiling, this operation must sometimes be repeated several times, and even under pressure, in order to kill all organisms originally present. Pasteur carried out a number of his experiments in glass flasks especially arranged with two necks (Fig. 32). One of the necks bore a short piece of rubber tubing, which was closed by a bit of glass rod. The other neck was drawn out into a narrow tube, bent twice upon itself. Both were open during the boiling of the liquid. While boiling was still going on the wide tube was plugged, after which boiling was stopped and the apparatus was cooled, air entering through the narrow tube. The solution remained unchanged indefinitely, since all spores contained in the entering air were caught in the narrow bend of the tube. However, if the glass stopper was momentarily removed, thus allowing a very small number of microörganisms to enter the flask, then the solution immediately began to decompose. Decomposition is brought about in such an experiment as a result of the rapid multiplication of the microörganisms that have been introduced.

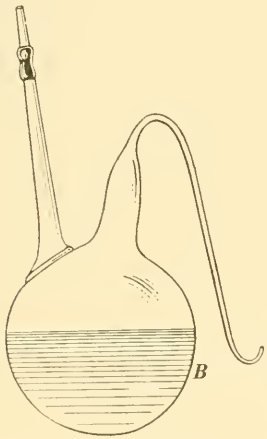


Fig. 32.—Pasteur flask.

To demonstrate conclusively that the theory of spontaneous generation is untenable, it remained still to prove that microörganisms and their spores really do occur in the air in great abundance. This question was also worked out by Pasteur in the most exact manner. He took a series of flasks, filled to a third of their volume with nutrient solution, brought the contents to boiling and then sealed them by fusing the glass of their narrow necks. The flasks were then placed in positions where he wished to investigate the air, and the sealed ends were then broken off, thus allowing air to enter. The flasks were then resealed. If the air entering a flask was free from germs, then the liquid remained unchanged, but if the entering air contained microörganisms or their spores, then decomposition began. In this way Pasteur proved that the air of deep cellars and high mountains is most nearly pure. It need not be concluded, however, that the air is absolutely free from organisms in those cases where the liquid remains unchanged in such experiments; it is quite possible that spores may be contained in the air but they may be able to develop in the particular nutrient medium chosen.

Many exact investigations have now been made upon the distribution of microörganisms in the air. The table given below presents the average results from ten years of observation (1885–1894) upon the number of microörganisms

in a cubic centimeter of air in the Park of Montsourie. In the same table are shown the corresponding numbers, averages from ten years of observations, in one of the squares in Paris (Place Saint-Gervais). The numbers are much larger in cities than in the country.

Season of Year	Park of Montsourie		Place Saint Gervais (Paris)	
	Bacteria	Moulds	Bacteria	Moulds
Winter	170	145	4305	1345
Spring	295	195	8080	2275
Summer	345	246	9845	2500
Autumn	195	230	5665	2185

Microörganisms occur not only in the air but also in water and soil. The water of rivers always contains bacteria, these being especially numerous in the vicinity of cities. The following numbers of bacteria were found in a cubic centimeter of water from the rivers and at the localities cited below.

River Rhone	above Lyons	75
	below Lyons	800
River Spree	above Berlin	4,300
	below Berlin	97,400

Microörganisms also occur in rain water, in snow and in hail.

The soil always contains microörganisms, their number naturally depending upon the amount of organic material present. Many more are found near the surface than in the deeper layers. The following table gives an idea of their distribution at various depths in a soil covered with forest growth (at Pfingstberg, in the vicinity of Potsdam). These are the numbers of microörganisms found in a cubic centimeter of soil from various depths at different times of the year.

Depth below Soil Surface, *meters*	May 27	June 15	Nov. 3
0.0	150,000	140,000	55,000
0.5	200,000	145,000	75,000
1.0	2,000	1,000	7,000
2.0	2,000	0	100
3.0	3,000	700	1,500
4.5	100	100	0

Bacteria are present in all foods, milk furnishing especially favorable conditions for their development. When fresh this liquid generally contains no bacteria, but they develop very quickly from spores that fall from the air. Thus a cubic centimeter of milk that had stood since milking at a temperature of 15.5°C., contained the following numbers of bacteria per cubic centimeter.

Hours after Milking	Bacteria per cc.
4	34,000
9	100,000
24	4,000,000

The intestinal tract of man is densely populated with bacteria, which frequently cause decomposition of foods in the intestine. We are thus not only externally surrounded by bacteria, but are even internally infested with them. This seems to explain why these organisms appear so promptly in all kinds of organic material that they decompose.

§5. **Sterlization and Disinfection.**[1]—In view of the fact that microörganisms are so universally present, all objects used in handling them must be absolutely free from spores or germs of any kind, especially if pure cultures of a certain species are desired. This is accomplished by sterilization. Such small objects

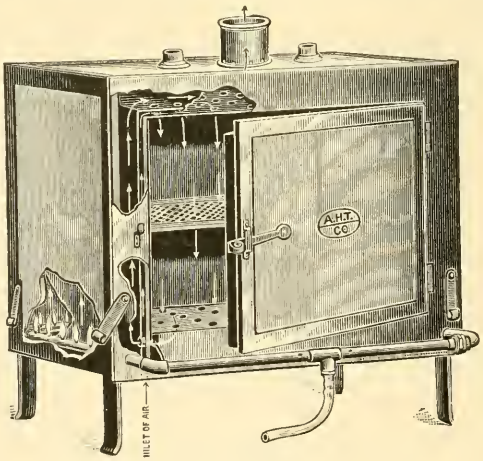


Fig. 33.—Dry-air sterilizer heated by gas.

as knives, scissors, glass rods, forceps, slides and cover glasses, platinum needles, etc., may be sterilized by heating in a gas or alcohol flame. Platinum instruments may be brought to a red heat but for other objects a few moments in the flame suffices, so that germs clinging to the surface may be destroyed. A drying oven, or dry-air sterilizer, is used for the sterilization of larger objects (Fig. 33). This is usually equipped with double walls, the products of combustion

[1] **Abel, Rudolf V. L.,** Taschenbuch für den bakteriologischen Praktikanten. [Abel's Laboratory handbook of bacteriology. Tr. from 10th German ed. by M. H. Gordon. London, 1907.] **Küster, Ernst,** Anleitung zur Kultur der Mikroörganismen für den Gebrauch in zoologischen, botanischen, medizinischen und landwirtschaftlichen Laboratorien. Leipzig and Berlin, 1907.

from the gas flame below passing between the two walls and thus rendering the heating uniform.[c]

Objects that cannot endure dry heat are sterilized in a steam sterilizer, such as Koch's apparatus. This is a cylinder of tinned sheet iron or copper with a cover above. The lower part is filled with water and the objects to be sterilized are placed upon a perforated rack in the upper part. A burner below the cylinder heats the water to boiling and the contained objects are sterilized by water vapor at 100°C. The apparatus is covered with felt or asbestos, to retard the escape of heat.[d]

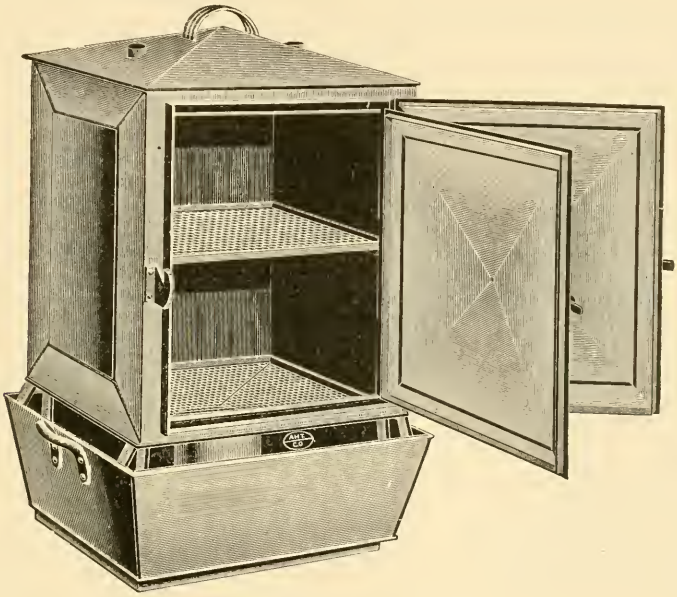


Fig. 34.—Arnold steam sterilizer.

Instead of a steam sterilizer the autoclave is frequently used for sterilization (Fig. 35). This is nothing more than a Papin's digester, operating with superheated steam, under pressure up to two atmospheres or more and at temperatures of from 100° to 134°C. or higher. At a temperature of 120°C. sterilization need last only fifteen minutes. At a temperature of 130° all germs are instantly killed, so that repeated treatment, necessary in the case of steam sterilization, is here superfluous.

[c] For most satisfactory work the oven should have an automatic temperature-regulator, various forms of which are available for gas. Electrically heated, automatically regulated ovens are also obtainable, some of which are so well insulated that but little heat escapes to the exterior.—*Ed.*

[d] One of the various forms of the Arnold type of steam sterilizer is most convenient and efficient in operation. (Fig. 34.) This keeps but a small amount of water boiling at any one time and a large portion of the water that is boiled away is condensed and returned to the reservoir.—*Ed.*

Liquids may also be sterilized by filtration. The most convenient arrangement for this purpose is the Chamberland filter, a hollow cylinder of porous porcelain, closed at one end. The liquid to be sterilized is passed, under pressure, through the porous walls of the previously sterilized filter.

Various disinfecting materials are also used for the chemical destruction of microörganisms. The most effective of these is corrosive sublimate, or mercuric chloride ($HgCl_2$). A solution of 1 g. of mercuric chloride in a liter of distilled water is thus used in bacteriological laboratories. The hands of the worker and also his implements are disinfected with this solution, which is also employed to destroy cultures that are not needed. A solution of one part of the salt in 300,000 parts of water prevents the development of the bacillus of splenic fever, *Bacillus anthracis*. Sulphurous acid, chlorinated lime [also known as bleaching powder; it contains calcium hypochlorite], hydrofluoric acid and its salts, boric acid, ozone, hydrogen peroxide, milk of lime, and phenol, or carbolic acid, are also suitable for use as disinfectants.[e]

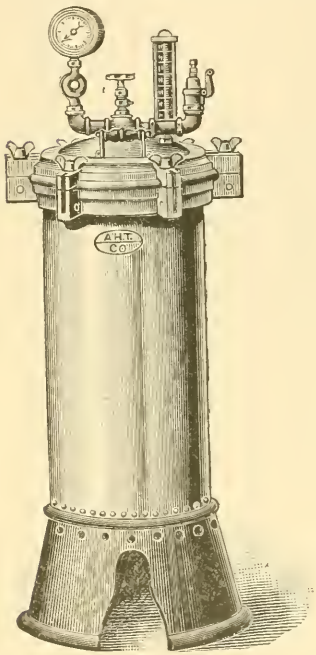


FIG. 35.—Autoclave. The top is hinged and may be raised after releasing the locking clamps.

§6. Pure Cultures.—To study microörganisms with respect to their developmental history and their physiological process it is necessary to obtain them in a pure culture.[1] A pure culture is one known to contain only a single, definite species of organism. Such a culture can be obtained only by fulfilling two conditions. The first consists in the exercise of sufficient precaution to prevent the entrance of germs from the air into the sterilized culture medium; the second is the derivation of the culture from a single cell. A culture in which all the microörganisms are quite similar is nevertheless not to be termed a pure culture unless it has been derived from a single cell, since very many microörganisms with entirely different physiological properties

[1] Pure cultures may be purchased from several establishments, among which may be mentioned the following: Krals Bakteriologisches Laboratorium, Prag I, Kleiner Ring II; Institut für Gärungsgewerbe, Berlin N, Seestrasse 65; Jörgensens Laboratorium, Kopenhagen, Frydendalsvej 30; Zentralstelle für Pilzkulturen, Amsterdam. [They may be obtained from the Laboratory of the American Museum of Natural History, New York, and from Parke, Davis and Co., Detroit.—*Ed.*]

[e] To the substances mentioned in the text may be added: iodine, sodium sulphite and Dakin's recent discovery, paratoluene-sodium-sulphochloramide (on the American market under the trade-name *chlorazene*, though it was called "chloramine" by Dakin [British med. jour., Aug. 25, 1915, also Jan. 29, 1916]). Chlorine, bromine, and potassium permanganate are also used as disinfectants. It should be noted, however, that antiseptics or disinfectants that are useful in some cases may be useless or even harmful in others. Numerous references on this subject are given in the Index Medicus, Carnegie Inst., Wash.—*Ed.*

have exactly the same form. On the other hand, a culture obtained from a single cell is called a pure culture, even though the microörganisms therein contained exhibit diverse forms, since we now know that one and the same species of bacterium or yeast can assume different forms, according to its developmental stage and the influence of the medium in which it is grown.

The method most frequently used for the production of pure cultures is that of dilution. This method was first used, in its original form, by Lister[1] in 1878, to obtain a pure culture of lactic acid bacteria. It was carefully elaborated for yeasts by the Danish bacteriologist, Hansen in 1881.[f]

Let it be supposed that we have a fermenting beer-wort with many different species of yeasts, and that these are to be separated, so that each species may be had in pure culture. After shaking the liquid, several drops are taken up in a sterilized pipette and transferred to a Freudenreich flask (Fig. 36) partly filled with sterilized water. This flask is of glass, with a capacity of from 25 to 30 cc., and is closed by means of a glass cap shaped like a short, inverted thistle-tube, the small opening of which is plugged with cotton. To obtain a uniform distribution of the yeast cells throughout the liquid, the flask is thoroughly shaken, after which a drop of the contents is transferred, upon the bent end of a platinum wire, to the surface of a microscope cover glass which is marked off into small squares. Here the drop is spread out into a thin layer, and the number of cells present is determined by counting. A van Tieghem cell, or moist chamber, is used for this purpose (Fig. 37). This consists of a slide upon which a glass ring (*c*) is sealed with vaseline. A small quantity of water (*d*) is introduced into the chamber so that microörganisms clinging to the under side of the cover glass (*a*) may not become desiccated. The cross-ruled cover glass is sealed to the glass ring with vaseline, the culture drop hanging from its lower surface (*b*). The divisions marked upon the cover glass facilitate the counting of the cells under the microscope.

Fig. 36.—Freudenreich flask.

Suppose that twenty cells are found upon the cover glass. The drop of liquid is again transferred, by means of the platinum hook, to a fresh Freudenreich flask containing 40 cc. of sterilized water. After vigorous shaking about 1 cc. of this liquid is transferred (with a pipette) into each of forty Freudenreich flasks containing sterilized beer-wort. Since the original drop contained only twenty cells, we should expect that the yeast would, in all probability, develop only in twenty of the flasks while the other twenty would remain sterile. It is also highly probable that the new generation has arisen from only a single cell in those flasks where growth does occur. All

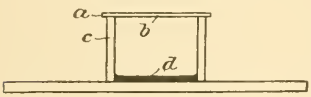


Fig. 37.—Moist chamber, or van Tieghem cell, for microscopic work. *a*, cover glass; *b*, position of drop of medium; *c*, wall of chamber made of section of glass tube; *d*, water or solution in bottom of chamber.

[1] Lister, Joseph, On the lactic fermentation and its bearings on pathology. Trans. Pathol. Soc. London 29: 425–467. 1878.

[f] Hansen, 1896. [See note 1, p. 44.].—*Ed.*

this is only highly probable, however, and not definitely established. Hansen employed this method in his work with yeasts. Flasks containing freshly inoculated beer-wort are vigorously shaken and then allowed to stand. The cells sink to the bottom and begin to multiply, so that, after a time, whitish colonies of cells become visible with the unaided eye. If a flask shows but one such colony it follows that only a single cell was introduced, since it is highly improbable that two cells might have settled together after the shaking. If, on the other hand, two or three cells have been introduced into the flask, then two or three colonies, respectively, develop.

In order to secure pure yeast cultures, solid substrata may also be employed, which make it possible to follow, under the microscope, the development of a colony from a single cell. For this purpose a drop from a young yeast culture—previously shaken—is introduced into a small flask of sterilized water. From this is inoculated, by means of the tip of a platinum wire, another flask containing beer-wort and gelatine, warmed to 45°C. The latter is vigorously shaken and then a drop of the liquid is transferred to a circular cover glass (30 mm. in diameter), which has been marked off into numbered squares, and the cover is laid over a glass ring to form a moist chamber or van Tieghem cell. The yeast cells are held immovable in the hardened gelatine so that it may now be

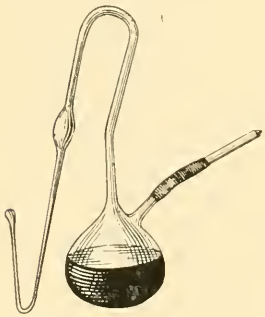


Fig. 38.—Pasteur flask; a slightly different form from that of Fig. 32, p. 54.

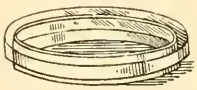


Fig. 39.—Petri dish.

Fig. 40.—Showing insertion of needle into solid medium in inverted tube, to make stab inoculation.

noted in which squares single ones lie, and the development of colonies from these may be readily followed. When the colonies become clearly visible to the unaided eye, one of them is removed from the cover glass and placed in a flask of nutrient solution. The colony is lifted on the end of a bit of flame-sterilized platinum wire, held by means of forceps, and the wire, with its colony, is dropped into the flask. During this operation the cover glass must be held with the drop on its under side, to prevent infection from the air. If a large quantity of pure culture is desired, a portion of a young culture a day old, obtained as just de-

scribed, is transferred with a pipette to a Pasteur flask (capacity about 200 cc.) of sterilized beer-wort (Fig. 38). After a day the contents of this flask are poured into second flask (capacity about 500 cc.) also filled with sterile beer-wort.

Solid as well as liquid nutrient media are used for pure cultures of bacteria. In the case of liquid media the dilution method described above is used to separate the cells. With solid media, which are very valuable for the production of pure cultures, Petri dishes are used for this purpose (Fig. 39). Each dish consists of two shallow glass pans (9 or 10 cm. in diameter), one being a little larger than the other and forming a cover for it. A trace of the mixed culture is introduced into a flask containing, for instance, a mixture of bouillon and gelatine, at 30°C., after which the flask is shaken, and the contents are then poured into the dish and the latter is covered. After some time each bacterial cell builds a colony around itself, which can be seen by the unaided eye or with a simple magnifying glass.

When a pure culture of a certain microörganism is finally obtained, then any number of pure cultures of that form may be readily prepared. Inoculations of liquid nutrient media are effected by means of a glass rod, a platinum wire or a pipette, with all the requisite precautions. Inoculations of solid media may take the form of either *stab* or *streak* cultures. To make a stab culture a platinum needle is dipped in the original culture and is then thrust upward into the solid medium held in an inverted test-tube (Fig. 40). For a streak culture, a test-tube of solid medium with a slanting surface is prepared, and the point of the inoculating needle is drawn across this surface.

Summary

1. **General.**—Plants without chlorophyll cannot form carbohydrates from carbon dioxide and water by means of the energy of sunlight. They derive energy, as well as material, from chemical compounds. Such plants may be divided into two groups: those of one group get energy from organic compounds alone (these compounds having been previously made by green plants), those of the other group derive energy from inorganic substances. Cells with chlorophyll utilize sunlight energy to form carbohydrates (and oxygen) out of carbon dioxide and water, while cells without chlorophyll either get carbohydrates (or related organic compounds) ready-made from their surroundings, being unable to utilize either sunlight energy or carbon dioxide, or else they derive energy from inorganic compounds and thereby form their carbohydrates and related compounds out of carbonates or carbon dioxide and water.

2. **Non-green Plants That Derive Energy Only from Organic Compounds.**—Yeasts, fungi, non-green seed plan s, the non-green portions of ordinary green plants, and most bacteria, derive their energy supply exclusively from ready-made organic compounds. These ompounds also supply carbon, which is of course as essential for non-green cells as for cells with chlorophyll.

The microörganisms of this group are very important in nature, being largely responsible for decay and putrefaction. They live by decomposing the organic substances produced by other organisms, including green plants. They may be distinguished from one another by the nature of the substances required for their growth, and they may be grown in artificial nutrient media, such as Pasteur's culture solution

for yeast. These organisms are either saprophytic (living on dead material from other organisms) or parasitic (living on tissues that are still alive). There are also a few saprophytes and parasites among flowering plants. Dodder (Cuscuta) is an example of a parasite of this kind. Mushrooms are examples of large saprophytic forms.

3. **Non-green Plants That Derive Energy from Inorganic Compounds.**—This group is composed of certain kinds of bacteria that are able to oxidize inorganic compounds and thus secure a supply of energy. Of these, nitrifying bacteria are very important. They oxidize ammonia to nitric acid. They must be grown in surroundings free from carbohydrates and other organic substances, but they require carbon dioxide (or carbonates) and oxygen. They form carbohydrates and other organic compounds out of water and carbonates or carbon dioxide, somewhat as do green plants, but their source of energy is very different. Another example of this group is furnished by the sulphur bacteria (as Beggiatoa), which oxidize hydrogen sulphide to sulphur and water, thus securing an energy supply. The sulphur produced is finally oxidized into sulphates, such as calcium sulphate. The sulphur bacteria grow in the presence of organic material. Some hydrogen bacteria (Hydrogenomonas) can form organic material from hydrogen, oxygen, and carbon dioxide, in the absence of organic compounds. Hydrogen is oxidized, thus supplying energy. In the presence of organic compounds hydrogen is not oxidized, and these bacteria are then to be considered as belonging to the preceding group.

This whole matter of the carbon nutrition of plants may be stated as follows: Apparently all organic compounds in plants are formed, directly or indirectly, from carbohydrates (such as sugars). (1) The carbohydrates used may be formed *in cells with chlorophyll*, out of carbon dioxide and water, and by means of sunlight energy (2) The carbohydrates used may be formed *in cells without chlorophyll*, out of carbon dioxide (or carbonates) and water, by means of energy obtained through the oxidation of inorganic substances such as ammonia, sulphur dioxide, hydrogen, etc. (3) The carbohydrates used may be derived from the surroundings, either ready-made or else by the decomposition of other organic compounds that are themselves supplied ready-made in the surroundings. These other organic compounds may also be used directly, without the preliminary step of forming carbohydrates. There are just two general sources of energy for plant activities, (*a*) sunlight and (*b*) energy derived from the oxidation of substances; and the substances oxidized may be either organic or inorganic.

4. **Microörganisms in Nature.**—Since Spallanzani's time it has been known that all organisms are formed by the reproduction of other organisms, and that the microörganisms found everywhere in nature arise in this way. On the basis of this principle Appert originated the art of preserving foods by sterilization. If all organisms in a preparation are killed at the start, and if no more are allowed to enter from without, there will be no living ones in the preparation. Fermentation and the decay of foods are caused by microörganisms, and these substances may therefore be preserved by sterilizing and then hermetically sealing them. This whole proposition was finally clearly worked out by Pasteur, who showed, among many other things, that the microörganisms that cause fermentation in foods, etc., originate from individuals of the same forms, which fall in from the air, etc. The air generally contains large numbers and many kinds of microörganisms as do also soil, water, the human alimentary tract, etc.

5. **Sterilization and Disinfection.**—To obtain objects or material absolutely free from living microörganisms sterilization is necessary. In many cases this is done by dry heat. In other cases steam is used, especially in a closed chamber, such as the

autoclave. The heat must be applied for an adequate period, and the temperature must be sufficiently high. Liquids are frequently sterilized by passing them through a suitable filter (such as the Chamberland), which retains the bacteria, etc. Sterilization may also be accomplished by the use of antiseptics or disinfectants, such as mercuric bichloride, phenol, etc. These simply poison the microörganisms.

6. Pure Cultures.—Pure cultures of any given kind of microörganism may be obtained by inoculating a suitable sterile medium with a single cell of the form desired, and allowing this to develop without the entrance of any other cells. Unless obtained in this way, a culture cannot be surely considered as pure. Single-spore inoculation is generally accomplished by repeated dilution of a liquid medium that contains the particular form desired. For this sort of work special technique has been devised.

CHAPTER III
ASSIMILATION OF NITROGEN[1]

§1. **The Nitrogen of the Air.**—Atmospheric air is fourth-fifths free nitrogen and it contains very small amounts of ammonia. We owe the first experiments upon the assimilation of free nitrogen to Boussingault,[a] who grew various plants from the seed in nitrogen-free, ignited sand to which was added some ash from seeds of the kind of plants employed. He placed the porous culture pot in a shallow glass dish supported above the bottom of a larger glass pan, in which stood a large bell-jar, covering the cultures. (See Fig. 41.) Some sulphuric acid was placed in the large pan, to prevent the entrance of ammonia from the outside air into the bell-jar. Two glass tubes were introduced under each jar, one to supply distilled water[b] to the dish in which the pot stood, the other to provide the necessary carbon dioxide to the air-space within the bell-jar. There was thus no source of nitrogen within the bell-jar, other than the free nitrogen of the air. The amount of nitrogen in the seed was determined, at the beginning of the experiment, by analysis of a control portion of the same kind of seed. The apparatus was exposed to light, and at the close of the experiment (after two or three months) the nitrogen content of the mature plant was determined, and no increase in this element could be detected. It follows from this that free nitrogen is not assimilated by ordinary higher plants when these are cultivated in the soil without microörganisms.

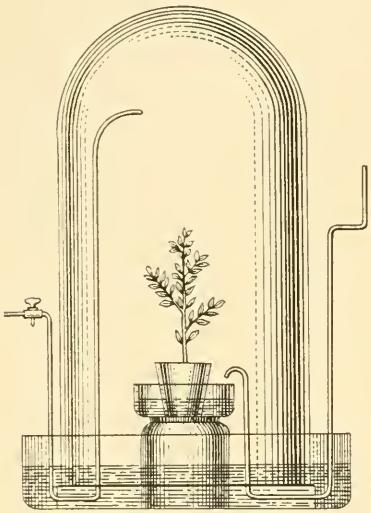


Fig. 41.—Arrangement of Boussingault, for growing a plant in nitrogen-free soil, without access of ammonia from the air. The large pan contains sulphuric acid (forming a seal); water is supplied through the tube at the right and carbon dioxide through the one at the left.

[1] A complete summary of the work upon nitrogen assimilation up to 1879 is given in: **Grandeau, L.**, Cours d'agriculture de l'école forestière. Chimie et physiologie appliés à l'agriculture et à la sylviculture I. La nutrition de la plante. Paris, 1879.*

[a] **Boussingault,** 1860–91. [see note 5, p. 2.] **Idem,** De l'action du salpêtre sur la végétation. Ann. sci. nat. Bot. *IV*, **4**: 32–46. 1855. **Idem,** Recherches sur l'influence que l'azote assimilable des engrais exerce sur la production de la matière végétale. *Ibid. IV*, **7**: 5–20. 1857.—*Ed.*

[b] It should be mentioned, however, that, while distilled water should not *add* anything but water and the atmospheric gases to the organism, yet it may *extract* other materials. Thus seedlings grown in distilled water give off salts, etc., by diffusion into the surrounding medium. (See, further, note *b*, p. 83.)—*Ed.*

Experiments upon the assimilation of ammonia from the air by leaves were carried out by Sachs,[c] by Schlösing[d] and by Adolf Mayer.[e] The upper parts of the plant were isolated from the soil and received the ammonia as the carbonate, in solution. All the plant parts so treated exhibited a higher nitrogen content than the corresponding organs in the controls without ammonia thus supplied. This kind of nitrogen assimilation is of almost no importance under natural conditions, however, since the ammonia content of the air is exceedingly small. According to Schlösing a volume of 100 cu. m. of air contains, on the average, only 2.4 mg. of ammonia.

§2. **The Nitrogen of the Soil.**—The nitrogen of the soil occurs as organic compounds, ammonium salts and nitrates.[f] The experiments of Boussingault and those of many agricultural chemists have shown that ordinary plants (with the exception of certain forms, especially the legumes, which will be discussed later) obtain their nitrogen exclusively from the soil, and that all three kinds of nitrogen compounds of the soil are beneficial to plants. Soils poor in nitrogen, and thus unproductive, can often be made productive by addition of any of these three forms of nitrogen compounds, but this result can usually be best and most quickly attained by the addition of nitrates. Therefore, the various nitrates generally serve as the best source of nitrogen for higher plants.

The question arises whether all nitrogen compounds of the soil are taken up directly by the plant or first undergo some alteration. In order to answer this question we must consider some of the properties of soils.

According to Boussingault 1 kg. of soil contained the following amounts of nitrogen:

Kind of N-compound	Source of Soil		
	Liebfrauenberg	Nancy	Mettais
	grams	*grams*	*grams*
Organic nitrogen	2.101	1.432	1.223
Nitrogen of ammonium salts	0.019	0.004	0.004
Nitrate nitrogen	0.029	0.040	0.055

Most of the soil nitrogen thus has the form of organic compounds, which are decomposition products from the decay of animal and plant materials. The

[c] **Sachs, J.,** as cited by **Robert Hoffman,** Ueber die Aufnahme des Kohlensäuren Ammoniaks der Luft durch die Pflanzenblätter. Jahresb. Agrikulturchem. **3**: 78–80. 1862.—*Ed.*

[d] **Mayer, Adolf,** Ueber die Aufnahme von Ammoniak durch oberirdische Pflanzentheile. Landw. Versuchsst. **17**: 329–397. 1874.—*Ed.*

[e] **Schloesing, Th.,** Sur l'absorption de l'ammoniaque de l'air par les végétaux. Compt. rend. Paris **78**: 1700–1703. 1874. Also see: **Atwater, W. O.** Ueber die Assimilation von Stickstoff aus der Atmosphäre durch die Blätter der Pflanzen. Landw. Jahrb. **14**: 621–632. 1885.—*Ed.*

[f] Nitrites also occur, but in small amount.—*Ed.*

nitrogen of ammonium salts forms the smallest part. The ammonia of the soil is derived partly from the decomposition or organic nitrogenous compounds and partly from the air. According to Schlösing's investigations, ammonia gas is vigorously absorbed from the air by both dry and moist soils. Dry soils, it is true, soon become saturated with ammonia, but this is not so for moist soils, for the ammonia absorbed is gradually converted into nitric acid. A soil surface of 1 hectare (2.5 acres) can absorb yearly from 53 to 63 kg. of ammonia from the air.

Besides organic compounds and ammonia, every soil also contains nitric acid or its salts. According to Boussingault's exact investigations nitric acid is formed in the soil at the expense of other nitrogenous compounds. A known quantity of damp soil, of known composition, was placed in a large carboy, which was sealed in 1859 and not reopened until 1871. At the conclusion of the experiment the soil in the carboy was again analyzed. The results are presented in the following table.

Year	Total nitrogen	Nitric acid	Nitric acid nitrogen
	grams	*grams*	*grams*
1859	0.4722	0.0029	0.00075
1871	0.4520	0.6178	0.16000
Difference.....	−0.0202	+0.6149	+0.15925

The nitric acid was at least mainly formed from other nitrogenous compounds present in the soil. Moreover, during the progress of the experiment a part of the nitrogen of the soil diffused into the air of the enclosed space. Boussingault showed in later experiments that very many kinds of organic materials (*e.g.*, meat, blood, horn, bone, wool, etc.), if added to the soil, serve as sources for the formation of nitrates. Conditions thus exist in the soil which render possible the transformation of a great many kinds of nitrogen compounds into nitric acid or nitrates.

Now the question arises, how is it that, in spite of the continuous formation of nitric acid, there is never more than a small quantity of this substance present in the soil? An answer is obtained from a consideration of the phenomena of absorption of various compounds by the soil.[g] The soil takes substances out of solution and retains them, so that a solution filtered through a soil layer becomes less concentrated. The first investigator to direct his attention to this phenomenon and to recognize its importance in agriculture was Bronner (1836), who describes the following experiment. A bottle with a small opening in the bottom is filled with fine sand or with half-dry, sifted garden-soil. Dark ill-smelling manure extract is gradually poured into the bottle until the entire soil-mass is saturated. The liquid issuing below is almost entirely odorless and

[g] This is partly the phenomenon now generally termed *adsorption*.—*Ed.*

colorless and has lost all the readily recognizable characteristics of manure extract.

More exact studies show that not all compounds are thus retained by the soil; while ammonium salts are absorbed, nitrates easily pass through. This characteristic of nitrates, their ability to be washed out of soils, explains the small nitrate content of the soil. All of the nitrates not absorbed by plants are washed down by the rain into the deeper soil layers. Of all the nitrogenous substances occurring in the soil, the organic materials and ammonium salts form, so to speak, the nitrogen stock of the soil. These are firmly held and so act as a constant source of nitrates, which may be absorbed by plant roots.

The investigations of Kostychev[1] have shown that organic nitrogenous compounds of humus do not consist solely of decomposition products of plant and animal substances but are mainly proteins, such as are the constituents of living organisms. In the leaf-mould formed by oak leaves that had been decomposing for twelve months the nitrogen content was 2.98 per cent., of which 2.73 per cent. was protein nitrogen and only 0.25 per cent. was made up of simpler nitrogenous compounds. These experiments constitute a new proof that the processes going on in the soil are not exclusively chemical, without the intervention of living cells, but are also physiological in their nature, being connected with the life-processes of organisms. The same author has shown that the phosphorus of the soil appears mainly in complex organic compounds such as are constituents of the lowest organisms. By virtue of its abundant bacterial life, the soil is practically a living mass.[h]

§3. **Nitrification in Soils.**—The ability of the soil to produce nitric acid or nitrates from various more complex nitrogenous compounds depends upon various conditions. One of these, according to Schlösing, is free access of oxygen. Equal amounts of the same soil were confined in five vessels, and a current of gas was passed through each vessel. The gas passed through the first vessel was pure nitrogen, so that this soil was without oxygen. The other vessels, II, III, IV and V, received mixtures of nitrogen and oxygen containing 6, 11, 16, and 21 per cent. of the latter gas, respectively. The amount of nitrate present in the soil was determined for each vessel at the beginning and end of the experiment. The results of these determinations, expressed as nitric

[1] **Kostytschew, P.**, Ueber die Mikroörganismen des Bodens. Kurlandische Land- und Forstwirtsch. Zeitg. (Riga) **5**: 13–14. 1890.

[h] On the nature of the organic matter of the soil see the following: **Schreiner, Oswald,** and **Shorey, Edmund C.**, The isolation of harmful organic substances from soils. U. S. Dept. Agric., Bur. Soils, Bull. 53. 53 p. Washington, 1909. **Idem,** Chemical nature of soil organic matter. *Ibid.* Bull. 74, 48 p. Washington, 1910. **Schreiner, Oswald,** and **Skinner, J. J.**, Nitrogenous soil constituents and their bearing on soil fertility. *Ibid.* Bull. 87. 84 p. Washington, 1912. **Trusov, A.**, The formation of humus by means of vegetable substances. [Russian.] Selskoie khoziaistvo i liesovodstvo (Economie agricole et sylviculture) Petrograd **246**: 233–245. 1914. Rev. in: Month. bull. agric. intell. and pl. diseases **6**: 540–541. 1915. Also rev. in: Exp. sta. rec. **34**: 619. 1916. **Idem,** same title. [Russian.] Selskoie khoziaistvo i liesovodstvo (Economic agricole et sylviculture) Petrograd **248**: 409–437. 1915. Rev. in: Month. bull. agric. intell. and pl. diseases **7**: 46–47. 1916. Also rev. in: Exp. sta. rec. **34**: 516. 1916.—*Ed.*

acid, are presented below. The soil without oxygen thus lost its whole content of nitrate, and those supplied with oxygen formed additional amounts, the quantity formed increasing with the amount of oxygen supplied.

Vessel Number and Oxygen Content of Gas	Nitric Acid Present in the Soil		Loss	Gain
	Nov. 18, 1872	July 3, 1873		
	mg.	*mg.*	*mg.*	*mg.*
I. No oxygen	64	00	64	...
II. 6 per cent. oxygen	64	263	...	199
III. 11 per cent. oxygen	64	286	...	222
IV. 16 per cent. oxygen	64	267	...	203
V. 21 per cent. oxygen	64	289	...	225

Nitrification in soils is due to bacterial action, as Schlösing and Müntz[1] have shown. These authors took a large-bore glass tube a meter long, filled it with a mixture of sand and lime and allowed sewage water containing ammonia to percolate slowly through it. After some days nitrate could be indentified in the filtrate. The ammonia of the water was oxidized in its passage through the tube. They also subjected the soil contained in the tube to the action of chloroform vapor during the percolation, to determine whether this oxidation was effected by the soil itself or by microörganisms contained therein. The result was a cessation of nitrification, the filtrate containing ammonia instead of nitrates in this case. Since the chloroform probably only repressed the vitality of the soil bacteria, without influencing purely chemical processes, Schlösing and Müntz concluded that the process of nitrification in the soil is caused by bacteria.

After many investigators had vainly endeavored to obtain the nitrifying bacteria of soil in the pure culture, Vinogradskii[2] was at length successful in this, as have been mentioned above (page 48).

Further investigations by Vinogradskii showed that the nitrification of ammonia and ammonium salts to nitrates is effected in the soil not by one but by two species of bacteria. One form produces nitrites (NO_2) from ammonium salts, and the other produces nitrates from nitrites. Vinogradskii proposed to reserve the term *Nitrobacteria* for all those bacteria that have to do with converting ammonium into nitrate. Investigation of the morphological characteristics of *nitrite-formers* from different sources shows that they belong to different species. The difference between the nitrite-formers of

[1] [Schloesing, Th., and Müntz, A., Sur la nitrification par les ferments organisés. Compt. rend. Paris 84: 301–303. 1877. Idem, same title. *Ibid.* 85: 1018–1020. 1877. Idem, same title. *Ibid.* 86: 892–895. 1878.]

[2] Winogradsky, S., Recherches sur les organismes de la nitrification. I, II, III, IV and V. 1890. [See note 1, p. 48.] Idem. Contributions à la morphologie des organismes de la nitrification. [Russian and French.] Arch. sci. biol. St.-Pétersbourg 1: 87–137. 1892.

the Old World and of the New is so great that it has even been necessary to distinguish two different genera, each with several species. The nitrite bacteria of the Old World constitute the genus *Nitrosomonas*, with two species (*N. europæa*, *N. javanensis*) and local varieties. Those of the New World form the genus *Nitrosococcus*. A third genus, *Nitrobacter*,[1] includes those bacteria that oxidize nitrites to nitrates.

The work of Vinogradskii led to the supposition that these organisms might get their carbon as magnesium carbonate, but Godlewski[2] showed that such is not the case. Even with magnesium carbonate ($MgCO_3$) present, no carbon assimilation occurs in an atmosphere devoid of carbon dioxide. The nitrifying bacteria are thus shown to obtain their carbon from the carbon dioxide of the air.

Further investigations of Vinogradskii and Omelianskii[3] cleared up the relation of nitrifying organisms to various organic compounds that check their growth. In the following table are given, for each of the two kinds of bacteria and for several organic compounds, the concentrations of the latter that just begin to retard growth and those that check it completely.

	Nitrite Formers		Nitrate Formers	
	Concentration		Concentration	
	Just Retarding Growth	Inhibiting Growth	Just Retarding Growth	Inhibiting Growth
Glucose	0.025–0.050	0.2	0.05	0.2–0.3
Peptone	0.025	0.2	0.8	1.25
Asparagin	0.05	0.3	0.05	0.5–1.0
Ammonia	.....	.....	0.0005	0.015

Vinogradskii and Omelianskii state: "The action of the above-named substances, in preventing nitrification, is so pronounced and becomes evident at such low concentrations, that these substances are not to be considered even as neutral in this case, although they are usually regarded as nutrients in bacteriology; on the contrary, their action is quite analogous to that of the substances that are known as antiseptics."

If the presence of organic substances checks the process of nitrification, then no nitrifying of organic nitrogenous compounds is to be expected in pure cultures of nitrobacteria. According to Omelianskii[4] these organisms are entirely lacking in ability either to break down organic nitrigenous compounds by splitting off ammonia, or to oxidize the nitrogen of these compounds directly. Or-

[1] On methods for pure cultures of nitrifying bacteria, see: **Oméliansky**, 1899. [See note 1, p. 49.]

[2] **Godlewski, Emil,** O nitryfikacyi ammoniaku. Krakow. 1896.*

[3] **Winogradsky, S.,** and **Oméliansky, V.,** L'influence des substances organiques sur le travail des microbes nitrificateurs. Arch. sci. biol. St.-Pétersbourg 7: 233–271. 1899.

[4] **Oméliansky, V.,** Sur la nitrification de l'azote organique. Arch. sci. biol. St.-Pétersbourg 7: 272–290. 1899.

ganic nitrogen can be nitrified by nitrobacteria only after it has been changed into ammonia or ammonium salts. The coöperation is thus necessary, of at least one of the bacterial forms that give rise to ammonia from organic compounds. Omelianskii was able to obtain nitrification of bouillon if he inoculated the medium with three species of bacteria at the same time: *Bacillus ramosus*, *Nitrosomonas* and *Nitrobacter*. If only *B. ramosus* and *Nitrosomonas* are introduced the process is limited to the formation of nitrous acid (nitrites), while *B. ramosus* and *Nitrobacter* produce only ammonia. Inoculation with *Nitrosomonas* and *Nitrobacter* leaves the bouillon unchanged. All these relations may be shown by a diagram, reproduced below, in which the bacteria that decompose organic compounds to form ammonia are represented by *a*, those that form nitrites are represented by *b*, and those that oxidize nitrous to nitric acid (nitrites to nitrates) are represented by *c*.

	Organic Nitrogen	Ammonia Nitrogen	Nitrite Nitrogen	Nitrate Nitrogen
$a + b + c$	→	→	→	→
$a + b$	→	→	→	
$a + c$	→	→		
$b + c$	No alteration of organic nitrogen.			

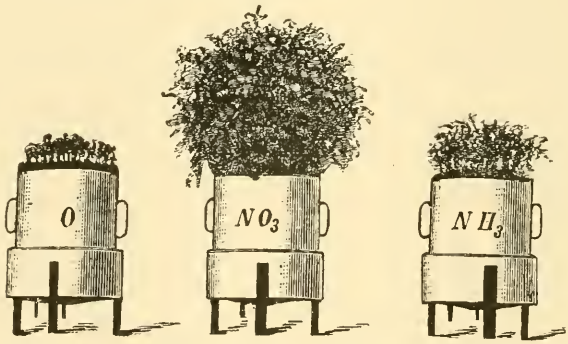


Fig. 42.—Comparison of the effect of nitrate and of ammonium salts on growth of plants in bog-soil, which is poor in lime. *O*, no fertilizer; NO_3, nitrate added; NH_3, ammonium salts added. (*After P. Wagner.*)

Now that we have become acquainted with the process of nitrification, we may consider the question whether higher plants are able to obtain their nitrogen only as nitrates or whether they can assimilate ammonium salts directly, without previous nitrification of the latter. Recent discoveries favor the view that nitrates act chiefly, if not exclusively, as the source of nitrogen for such plants. The experiments of Wagner[1] have shown that nitrates and ammonium salts have different effects according to the nature of the soil employed. Turnips were grown in vessels of a bog-soil very poor in calcium. In one series of experiments some of the vessels contained no nitrogen fertilizer, others each

[1] **Wagner, Paul,** Düngungsfragen unter Berücksichtigung neuer Forschungsergebnisse. Heft. IV. Berlin, 1898. 72 p.

contained 2 g. of nitrogen as nitrates, and still others each contained about 2 g. of nitrogen as ammonium salts. In a second series calcareous marl was added throughout, in addition to the fertilizers mentioned above. The results of this experiment are brought together in the following table (see also Fig. 42).

	Fertilizer	Dry Yield	Increase in Yield, Compared with Culture without Nitrogen
		grams	*grams*
Without lime	Without addition of nitrogen	6.3	....
	2 g. of nitrogen as nitrate	94.4	88.1
	2 g. of nitrogen as ammonium salt	29.4	23.1
With lime	Without addition of nitrogen	9.6	....
	2 g. of nitrogen as nitrate	92.0	82.4
	2 g. of nitrogen as ammonium salt	86.7	77.1

Thus, ammonium salts have but little value as fertilizers for soils poor in lime. But soils rich in lime show almost as good yields with ammonium salts as with nitrates (Fig. 43). These experiments show that nitrate fertilizer is suitable

Fig. 43.—Comparison of the effect of nitrate and ammonium salts on growth of plants in soil rich in lime. *O*, no fertilizer; NO_3, nitrate added; NH_3, ammonium salts added. (*After P. Wagner.*)

for many different kinds of soils whereas ammonia fertilizer is suitable for only a limited number. There are two reasons for this: first, if we suppose that the ammonia is all oxidized to nitric acid before assimilation, then free nitric acid may be produced in the soil that lacks calcium (as in the first series of experiments just described), and this acid retards the growth of the plants as well as the nitrification process. Secondly, if we suppose that a part of the ammonia is assimilated unchanged, then free acid may again accumulate in the soil lacking calcium; for ammonium salts are physiologically acid, their basic radicals being absorbed by the plants to a greater extent than are their acid radicals.[1]

[1] This is more fully considered in Chapter IV.

The presence of calcium carbonate prevents the accumulation of free acid in both cases.

Such experiments with natural soils cannot answer the question regarding the direct assimilation of ammonia. Sterilized soils must be used, in which the nitrifying process is eliminated. The experiments of Pitsch,[1] Breal,[2] and Kossovich,[3] who used sterilized soils, gave positive results.

§4. Circulation of Nitrogen in Nature.—The investigations of Boussingault and of Schlösing and Müntz [see note *a*, page 64, and notes *c*, *d*, *e*, page 65] established the view that higher plants can assimilate only combined nitrogen. Free nitrogen should thus have absolutely no value for green plants, in spite of the enormous amount of it present in the air. Schlösing pictures the circulation of nitrogen in nature in the following way. Nitric acid (HNO_3) formed in the soil is taken up by plants and transformed into proteins and other organic compounds, which, in their turn, serve for the nutrition of animals. These compounds of nitrogen finally return to the soil as decomposition products of plants and animals, and are there again oxidized to nitrates. The nitrate of the soil, that is not assimilated by plants, is washed into the deep soil layers by precipitation water and finally reaches the sea, where it is changed back into ammonium salts by the life activities of marine organisms. Ammonia evaporates, with water vapor, from the surface of the sea, and is again taken up from the atmosphere by plant leaves or by the soil, and in this way re-enters the general circulation. All these transformations of combined nitrogen have no effect upon the total amount of it occurring in nature. Natural processes are known, however, which lead to the decomposition of nitrogenous compounds and to the liberation of molecular nitrogen. Thus, in the complete combustion of nitrogenous organic compounds the total nitrogen is eliminated as nitrogen gas. The decomposition of organic compounds in the soil is also accompanied by the liberation of free nitrogen.

The total amount of combined nitrogen in nature is diminished by these processes and, for this reason, many authors have sought some natural process that might lead to fixation of free nitrogen. Nitrogen is one of the elements that form only weak combinations with other elements. Until recently chemistry could name but three kinds of nitrogen fixation that might be of importance in nature: (1) An electric spark discharge effects the union of nitrogen and oxygen (Cavendish). (2) A silent electrical discharge causes the union of nitrogen with organic substances (Berthelot). (3) During the evaporation of water a small amount of nitrogen combines with hydrogen from the water and produces ammonium nitrate (Schönbein). Only the first of these three possibilities has real significance in nature, namely the fixation of atmospheric nitrogen during thunderstorms.

[1] **Pitsch, Otto,** Versuche zur Entscheidung der Frage ob saltpetersäure Salze für die Entwicklung der landw. Kulturgewächse unentbehrlich sind. II. Landw. Versuchsst. 42: 1–95. 1893. **Pitsch, O.,** and **Haarst, J. Van,** same title as above, III. *Ibid.* 46: 357–382. 1896.

[2] **Bréal, E.,** Contribution à l'étude de l'alimentation azotée des végétaux. Ann. agron. 19: 274–293. 1893.

[3] **Kossowitch, P.,** Ammoniaksalze als unmittelbare Stickstoff Quelle für pflanzen. [Abstract in German, p. 637–638. Text in Russian.] Jour exp. Landw. 2: 625–638. 1901.

Recent technical advance has made it possible to obtain larger amounts of nitrogen compounds from atmospheric nitrogen. By oxidation of the latter, with the electric current, nitric acid is obtained on a large scale. By passing nitrogen through glowing calcium carbide, calcium cyanamide is formed, according to the equation: $CaC_2 + 2N = CaCN_2 + C$. The German commercial name of this product in the raw state is "Kalkstickstoff"[1] and it is used as a nitrogen fertilizer.

What has been attained by man only after much travail is commonly accomplished by plants, however, for we now know a number of plants that can assimilate atmospheric nitrogen.

§5. **Fixation of Atmospheric Nitrogen by the Leguminosæ.**—All legumes are able to develop normally, producing a rich harvest with a high nitrogen content, without the addition of any nitrogenous compounds to the soil, as the exact studies of Lawes and Gilbert[i] have shown. If we cultivate some sort of grain or legume for many years in succession on the same field without applying fertilizer, the nitrogen content of the crop finally reaches a certain minimum, beyond which it does not alter. Addition of mineral fertilizers without nitrogen is almost without effect upon the yield of grain, the nitrogen content remaining almost the same as before. This is entirely different in the case of the legumes; the same mineral fertilizer without nitrogen produces a marked increase in the nitrogen content of this crop.

Two series of experiments by P. Wagner[2] are illustrated in Figs. 44 and 45, one with peas and the other with oats, the experimental conditions being the same in both cases. The containers marked O contained no fertilizer at all, those marked KP contained potassium and phosphoric acid (PO_4), and those marked KPN contained potassium, phosphoric acid and nitrogen as nitrate. Comparison of these figures reveals a distinct difference between the legumes and the grains in their relation to fertilizers. The growth of oat plants is seen to be very slight in the unfertilized culture, and the addition of potassium and phosphoric acid produces no improvement; while the addition of these together with potassium nitrate produces excellent growth (Fig. 44). The behavior of the pea plants is entirely different. These do not need nitrate fertilizer, addition

[1] **Frank, A.,** Die Nutzbarmachung des freien Stickstoffs der Luft für Landwirtschaft und Industrie. Zeitsch. angew. Chem. **16**: 536–539. 1903. **Gerlach, M.,** Die Nutzbarmachung des atmosphärischen Stickstoffes. Illustr. landw. Zeitg. 1904. Nos. 5 and 7.* Review by Vogel in: Centralbl. Bakt. *II*, **12**: 495–497. 1904. [See also review in: Exp. sta. rec. **15**: 25. 1903–04.]

[2] **Wagner, P.,** Ergebnisse von Dünungungsversuchen in Lichtdruckbildern mit erläuterndem Vorträge über die rationelle Düngung der land wirtschaftlichen Kulturpflanzen. 2te Aufl. Darmstadt, 1891.

[i] **Lawes J. B., and Gilbert, J. H.,** The sources of the nitrogen of our leguminous crops. Jour. Roy. Agric. Soc. England *III*, **2**: 657–702. London, 1891. **Idem,** The Rothamsted memoirs on agricultural chemistry and physiology. 7 v. London, 1886–1899. **Idem,** same title. 3 v. London, 1890–1893. **Hall, A. D.,** The book of the Rothamsted experiments. 294 p. New York, 1905. For a brief discussion of this whole matter see: **Russell, E. J.,** Soil conditions and plant growth. 4 th ed. London and New York 406 p., 1921. Russell's excellent bibliography includes references to a number of the papers of Lawes and Gilbert. These papers have all been collected and published in the Rothamsted Memoirs, and Lawes and Gilbert's results are summarized by Hall.—*Ed.*

FIG. 44.—Growth of oats with various fertilizers. *O*, without addition to the soil; *KP* with addition of potassium and phosphoric acid; *KPN*, with addition of potassium, phosphoric acid and potassium nitrate. (*After P. Wagner.*) Compare with Fig. 45.

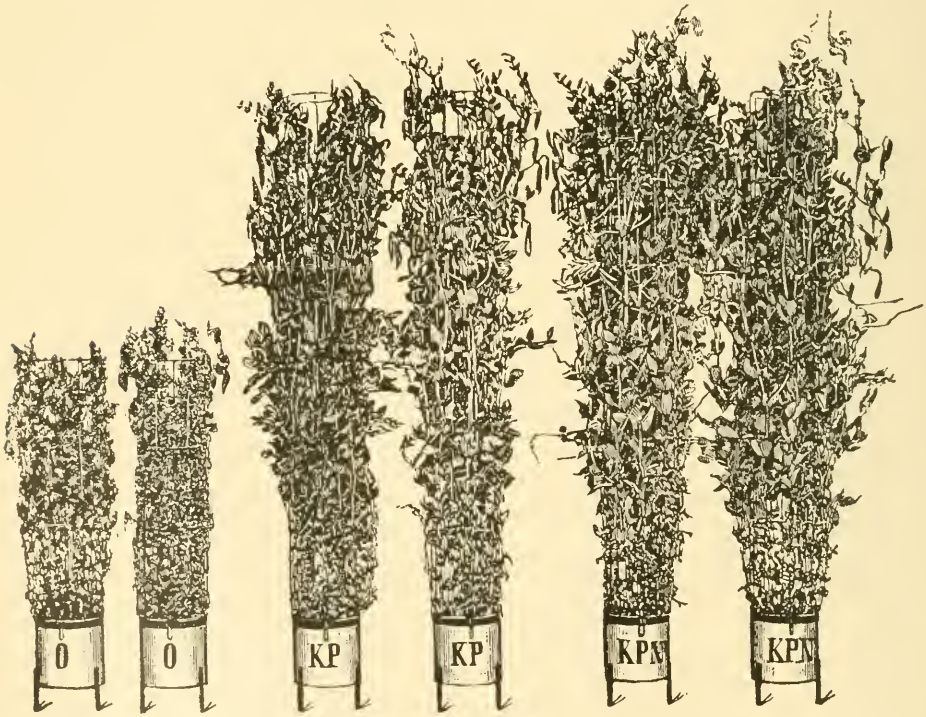


FIG. 45.—Growth of peas with various fertilizers. *O*, without addition to the soil; *KP*, with addition of potassium and phosphoric acid; *KPN*, with addition of potassium, phosphoric acid and potassium nitrate. (*After P. Wagner.*) Compare with Fig. 44.

of potassium and phosphoric acid being sufficient to produce normal growth. In this case the total need of nitrogen is supplied from the air (Fig. 45).

The results of Lawes and Gilbert and those of Wagner thus seem to disagree with the conclusions reached by Boussingault (see page 64). This is explained by the fact that Boussingault used sterilized soils, whereas the other authors, just referred to, worked with unsterilized soil under natural conditions. The reason for the entirely different behavior of legumes in sterilized soils and in unsterilized soils has been discovered in a series of remarkable investigations conducted by Hellriegel and Wilfarth.[1] In their experiments, various legumes grew quite normally in soils that lacked nitrogen, provided these soils were not previously sterilized. Growth was checked, however, in sterilized, nitrogen-free soils, because of lack of nitrogen. Addition to the sterilized soil of a small quantity of an infusion from unsterilized soil produced normal growth of the plants and resulted in a crop rich in nitrogen. If the added infusion was previously boiled, however, then its addition produced no effect at all; the plants were retarded in their development and the harvest showed no increase in nitrogen. The soil used in preparing the infusion must be taken from a field upon which plants of the kind used in the experiment have been cultivated; for example, if peas are employed the soil used for the water extract must be obtained from a field where peas have previously been grown.

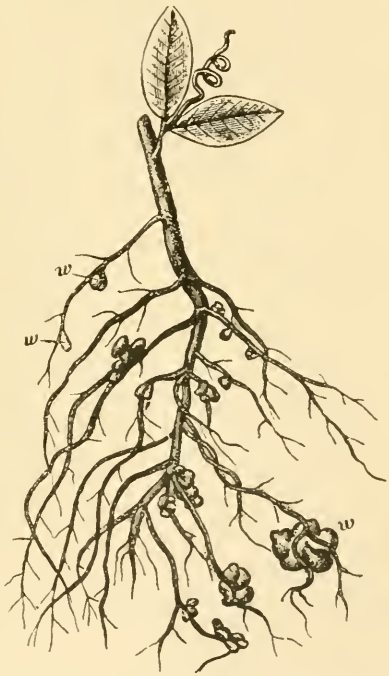


Fig. 46.—Root system of pea plant, with tubercles (*w*.)

Legumes growing under natural conditions have small tubercles upon their roots (Fig. 46). Hellriegel and Wilfarth observed that these tubercles developed only in unsterilized soil, or in sterilized soil only if it had been treated with infusion of unsterilized soil. Tubercles never develop in uninoculated sterilized soils.

From their studies Hellriegel and Wilfarth came to the conclusion that the formation of root tubercles is the result of a symbiosis between the legumes and lower organisms, and that these very tubercles are directly influential in the assimilation of atmospheric nitrogen by leguminous plants.

A cross-section of a legume root, through one of these tubercles, shows that the greater part of the tubercle consists of parenchymatous tissue (Fig. 47). The inner cells are very different from the outer ones. The former constitute

[1] **Hellriegel, H.,** and **Wilfarth, H.,** Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. Beilage Zeitschr. Rübenzucker-Indust. d. deutsch. Reich. 234 p. November, 1888.

the so-called bacterioid tissue and are characterized by thin cell walls and high content of protein. The protein substances occur in the cells as small, bacteria-like rods, which are branched in the older tubercles. These are the so-called bacterioids. The cells of the outer parenchyma layers contain little reserve material, and only those adjacent to the bacterioid tissue are filled with starch grains. The tubercle is covered on the outside by a layer of cork, and branches of the vascular bundles of the root extend into the tubercle.

Beijerinck[1] and Prazmovskii[2] have succeeded in securing tubercle bacteria in pure culture. When transferred to a nutrient solution, the young bacteria, or the modified cells called bacterioids, begin to divide and multiply rapidly. The newly formed organisms appear to be in no way different from ordinary bacteria, and they show the same kind of movement. Prazmovskii has given them the name *Bacterium radicicola*.

This writer has studied the developmental history of the tubercles of the pea plant. If sterilized soil in which young pea seedlings are growing is inoculated with a pure culture of *Bacterium radicicola*, an accumulation of bacteria in the root-hairs becomes noticeable after several days. This mass of bacteria then becomes enclosed in a sheath, forming a sack-like body that enlarges and

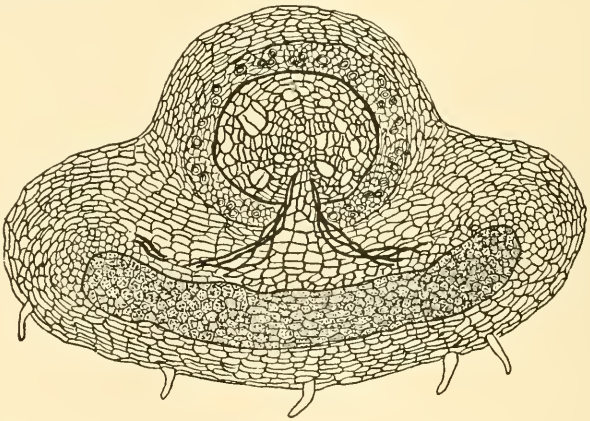


FIG. 47.—Cross-section of a root tubercle of lupine, showing bacterioid tissue (the elongated area below) surrounded by root parenchyma. The dark lines above the bacterioid area represent vessels that penetrate from the uninjured root to the hypertrophied tubercle.

penetrates through the root-hair into the root parenchyma as a bacterial filament. Having advanced into the root, this filament begins to branch rapidly. A lively division of the cells of the root parenchyma proceeds at the same time, in the neighborhood of the bacterial filament, which results in a swelling in this region of the root and in the formation of a tubercle. The branches of the filament occupy the central portion of the tubercle. The filament sheath finally disintegrates and the bacteria thus liberated enter the cell sap. Here they

[1] Beijerinck, M. W., Die Bacterien der Papilionaceen-Knöllchen. Bot. Zeitg. 46: 725–735, 741–750, 757–771, 781–790, 797–802. 1888.

[2] Prazmowski, Adam, Die Wurzelknöllchen der Erbse. I. Teil. Die Aetiologie und Entwickelungsgeschichte der Knöllchen. Landw. Versuchsst. 37: 161–238. 1890.

enlarge and become branched, thus becoming mature bacterioids. At this time the vascular bundles develop in the tubercle. The bacterioid tissue becomes depleted after a time, its contents being used up by the plant. The bacterial cells collect in groups in the remaining portions of the infection-filaments and become enclosed in a hard sheath. The spore-like colonies thus formed fall away after the destruction of the tubercle and are capable of infecting other roots the following spring.

Kossovich[1] sought to solve the question as to what organs of legumes absorb atmospheric nitrogen. He carried out two series of experiments, in one case depriving the leaves, and in the other case the roots, of nitrogen. He came to the conclusion that nitrogen is absorbed by the roots.

Infection of legumes with cultures of *Bacterium radicicola* does not always have a favorable influence upon the growth of these plants. If the inoculation occurs late in the growing season (in July), the result is an abundant formation of root tubercles, but the plants, instead of growing better, grow more poorly than do uninfected individuals. The action of the bacteria is merely parasitic in this case. Microscopic investigation shows that the transformation of bacteria into bacterioids does not occur here, and it was for this reason that Nobbe and Hiltner[2] believed assimilation of atmospheric nitrogen to be correlated with the formation of the bacterioids. Long-continued cultivation upon nutrient gelatine (from spring until midsummer) is said to make *Bacterium radicicola* more vigorous and to deter its transformation into bacterioids after it enters the root. Plants inoculated late in the season, being already partially exhausted at this time, are too weak to produce this change in the infecting organism.

Investigation of the tubercle bacteria of various legumes leads to the conclusion that there are many varieties of these organisms. In order to obtain a healthy development of *Robinia pseudacacia* in soil without available nitrogen, inoculations must be made with cultures from Robinia tubercles; infection with bacteria from pea and lupine tubercles has no effect at all. But inoculation with cultures from Cytisus tubercles has almost as good an effect as inoculation with cultures of the bacteria of Robinia itself.[3]

Certain non-leguminous plants also assimilate atmospheric nitrogen by symbiosis with bacteria, and the tubercles may be formed in other regions of the plant besides the root system. For example, the leaves of some of the tropical Rubiaceæ are characterized by numerous rounded, tubercle-like thickenings, which contain peculiar bacterial cells (*Mycobacterium rubiacearum*). These bacteria fix nitrogen from the air in the same general manner as do the root-tubercle bacteria of legumes.[4] (See Fig. 48.)

[1] **Kossowitsch, P.,** Durch welche Organe nehmen die Leguminosen den freien Stickstoff auf? Bot. Zeitg. 50: 697–702, 713–723, 729–738, 745–756, 771–774. 1892.

[2] **Nobbe, F.,** and **Hiltner, L.,** Wodurch werden die knöllchenbesitzenden Leguminosen befähigt, den freien atmosphärischen Stickstoff für sich zu verwerten? Landw. Versuchsst. 42: 459–478. 1893.

[3] **Nobbe, F., Schmid, E., Hiltner, L.,** and **Hotter, E.,** Versuche über die Stickstoff-Assimilation der Leguminosen. Landw. Versuchsst. 39: 327–359. 1891.

[4] [**Faber, F. C. von,** Das erbliche Zusammenleben von Bakterien und tropischen Pflanzen. Jahrb. wiss. Bot. 51: 285–375. 1912.]

§6. Assimilation of Atmospheric Nitrogen by Bacteria.—The work of Berthelot[1] rendered assimilation of free nitrogen by the bacteria of the soil very probable, but we owe the final solution of this problem to Vinogradskii[2] and Beijerinck.[3] Vinogradskii caused the development of nitrogen-fixing

FIG. 48.—*A*. Leaves of *Pavetta indica*, showing nodules, which contain nitrogen-fixing bacteria. *B*. Cells of *Mycobacterium rubiacearum* from leaf nodules of *Pavetta zimmermanniana*. Magnified to 3000 diameters. (*After Faber.*)

[1] **Berthelot, Marcellin,** Fixation de l'azote atmosphèrique sur la terre végétale. Ann. chim. et phys. 13: 5–14, 15–73, 74–78, 78–92, 93–119. 1888.

[2] **Winogradsky, S.,** Sur l'assimilation de l'azote gazeux de l'atmosphere par les microbes. Compt. rend. Paris 116: 1385–1388. 1893. **Idem,** same title. *Ibid.* 118: 353–355. 1894.

[3] **Beijerinck, M. W.,** Ueber oligonitrophile Mikroben. Centralbl. Bakt. *II*, 7: 561–582. 1901. **Freundenreich, Ed. von,** Ueber stickstoffbindende Bakterien. *Ibid. II*, 10: 514–522. 1903. **Löhnis, F.,** Beiträge zur Kenntnis der Stickstoffbakterien. *Ibid. II*, 14: 582–604, 713–723. 1905. **Christensen, Harald, R.,** Ueber das Vorkommen und die Verbreitung des Azotobacter chroococcum in verschiedenen Böden. Ein Beitrag zur Methodik der mikrobiologischen Bodenforschung. *Ibid. II*, 17: 109–119, 161–165, 378–383, 528. 1907. **Bredemann, G.** Regeneration der Fähigkeit zur Assimilation von freiem Stickstoff des Bacillus amylobacter A. M. et Bredemann und der zu dieser Spezies gehörenden bisher als Granulobacter, Clostridium usw. bezeichneten anaeroben Bakterien. Ber. Deutsch. Bot. Ges. 26: 362–367. 1908. **Idem.,** Bacillus amylobacter A. M. et Bredemann in morphologischer, physiologischer und systematitscher Beziehung. Mit besonderer Berücksichtigung des Stickstoffverbindungsvermögens dieser Spezies. Centralbl, Bakt. *II*, 23: 385–568. 1909.

microörganisms by inoculating a grape-sugar solution with garden soil. In spite of the fact that this solution contained no nitrogenous compounds, a vigorous fermentation began immediately, with the formation of carbon dioxide, much hydrogen, and butyric and acetic acids, the process being accompanied by the fixation of atmospheric nitrogen. The amount of nitrogen combined was related to the amount of sugar used up, as is shown in the following table:

Experiment number	1	2	3	4
Grams of sugar consumed	2.0	2.0	4.0	20.0
Milligrams of nitrogen fixed	3.9	5.9	9.7	28.0

Addition of ammonium salts in very small amounts acted favorably; larger amounts retarded the fixation of nitrogen and finally stopped it altogether. The fixation of atmospheric nitrogen is possible only in substrata which are either entirely deficient in nitrogenous compounds or contain these only in very small amounts. The bacterium to which this fixation is due was named by its discoverer, Vinogradskii, *Clostridium pasteurianum*. It is anaerobic, living without free oxygen.

More recently Beijerinck has found another nitrogen-fixing bacterium, *Azotobacter chroococum*. Unlike the forms previously mentioned, this is aerobic and thrives best in the presence of air, where it also exhibits its ability to fix nitrogen. Furthermore, other investigators have found other soil microörganisms that possess, to a smaller degree, this power to assimilate free nitrogen. The fixation of atmospheric nitrogen is therefore a process that occurs commonly in nature.

§7. Assimilation of Nitrogen Compounds by Lower Plants.—We have seen that nitrates usually furnish the best source of nitrogen for higher plants. Of the lower plants without chlorophyll (moulds, yeasts, bacteria) not nearly all are capable of utilizing nitrates. To be sure, this property is possessed by most of the common moulds (Penicillium, Aspergillus and some species of Mucor) and one group of bacteria is sufficiently specialized to utilize nitrates as a source of nitrogen, at the same time reducing them vigorously, with elimination of free nitrogen (denitrifying bacteria[1]). Nevertheless, most lower plants require organic nitrogenous substances, or at least ammonium salts. Suitable culture media for such forms have already been referred to, and it has also been mentioned that these organisms are in great variety, as far as their nutrition is concerned.

Summary

1. The Nitrogen of the Air.—By volume measurement, about four-fifths of the air is free nitrogen. Air also generally contains very small amounts of nitrogen in the form of ammonia. Free nitrogen cannot be assimilated by ordinary higher plants. Under natural conditions these plants may assimilate minute quantities of nitrogen from the ammonia of the air, but this source of nitrogen is generally quite negligible.

[1] **Laurent, E.**, Recherches sur le polymorphisme du *Cladosporium herbarum*. Ann. Inst. Pasteur 2: 558–566, 581–603. 1888. **Idem**, Recherches sur la valeur comparée des nitrates et des sels ammomiacaux comme aliment de la levure de bière et de quelques autres plantes. *Ibid.* 3: 362–374. 1889. **Ritter, G.**, Ammoniak und Nitrate als Stickstoffquelle für Schimmelpilze. Ber. Deutsch. Bot. Ges. 27: 582–588. 1909.

2. **The Nitrogen of the Soil.**—The soil contains free nitrogen (which cannot be assimilated by ordinary plants), ammonia and ammonium compounds, nitrates, nitrites, and organic nitrogenous substances. Nitrates in the soil are the main source of nitrogen for ordinary plants, though some forms are apparently able to assimilate some nitrogen in the form of nitrites or in the form of ammonia or ammonium salts. Organic nitrogen compounds are first decomposed (by soil microörganisms), giving nitrates, and then the resulting nitrates are assimilated by higher plants. Ammonium salts are similarly converted to nitrates in the soil, by microörganisms, as also are nitrites. Ammonium compounds and organic nitrogenous substances (arising from the decay of animal and plant tissues, etc.) are held in the soil in considerable amounts, but nitrates are readily washed out by percolating rain water, and carried away in the soil drainage. More nitrates are gradually formed, so that there is always a supply of these salts that may be absorbed through the roots of ordinary plants and assimilated.

3. **Nitrification in Soils.**—The production of nitrates in the soil, from other nitrogenous compounds (and from free nitrogen), occurs through the action of soil bacteria. For the activity of these nitrifying organisms a continuous supply of oxygen is necessary in the soil. According to Vinogradskii's work, ammonia and ammonium salts are assimilated by *nitrite bacteria* in the soil, which give off nitrites, and nitrites are assimilated by *nitrate bacteria* in the soil, which give off nitrates. These two groups of soil bacteria derive their carbon compounds by synthesis, from carbon dioxide or carbonates as source of carbon. The energy for this synthesis is derived from the oxidation of ammonia or of nitrites; the nitrites and nitrates that are produced may be considered as by-products. In the presence of organic compounds that may be readily oxidized (like sugars), these bacteria secure their carbon compounds directly, without synthesis from carbon dioxide, and they do not alter the organic nitrogenous compounds that may be present, nor does nitrification occur. Nitrogenous organic compounds are not assimilated by the nitrite and nitrate bacteria, but they are used by another group of soil bacteria, the ammonifying forms, which give off ammonia as a by-product. When bacteria of all three groups are present, the nitrogen of other nitrogenous compounds is ultimately converted, by three steps, into nitrate nitrogen. (1) The ammonifiers produce ammonium compounds (NH_4) from nitrogenous organic substances. (2) The nitrite bacteria produce nitrites (NO_2) from ammonium compounds. (3) The nitrate bacteria produce nitrates (NO_3) from nitrites.

Ammonium salts are generally not largely assimilated by ordinary plants, and ammonium nitrogen usually becomes readily assimilable only after nitrification, with formation of nitrates. Wagner found that ammonium salts were beneficial, as fertilizer, in lime soils, but not in other soils. In lime soils the lime prevents the development of any considerable acidity. Nitrites are generally not largely assimilated by ordinary plants.

4. [6] **Assimilation of Atmospheric Nitrogen by Soil Bacteria.**—Still another group of soil bacteria assimilate free nitrogen, as was shown by Vinogradskii and Beijerinck, and these bacteria form organic nitrogen compounds or nitrates. The energy for this nitrogen fixation is derived from the oxidation or fermentation of organic compounds, such as sugars. Some of these nitrogen-fixing bacteria thrive in the presence of oxygen, others are inhibited by oxygen. There are also soil bacteria, thriving under special conditions, that convert nitrate nitrogen into nitrite or ammonium nitrogen, or even into free nitrogen, these being denitrifying processes.

5. **Fixation of Free Nitrogen by Tubercle Bacteria.**—Although ordinary plants are not able to assimilate free nitrogen, there are certain groups of them (especially the

legumes) that appear to do so. Hellriegel and Wilfarth showed that the roots of legumes are normally infected with *nodule* or *tubercle bacteria*, which remain in the soil from season to season, infecting the new plants each year. The same experimenters showed that these microörganisms carry on nitrogen fixation in the structurally characteristic root-tubercles that result from their invasion of the legume root tissues. The tubercle bacteria apparently derive carbohydrates from the host, secure utilizable energy through the oxidation of these substances, and use some of this energy for the formation of nitrates or nitrogenous organic compounds from carbohydrates and free nitrogen. Nitrates, or organic nitrogenous compounds, are given off by the bacteria and these substances are assimilated by the host plant. Legumes may thus grow well in soils with very small supplies of nitrates, or none at all, deriving their nitrogen from the free nitrogen of the soil, through the activities of the tubercle bacteria. In the presence of considerable supplies of soil nitrates this fixation of free nitrogen is slight or does not occur. Different legumes have different nodule bacteria; the right form of the latter must be present in the soil for any given legume. Free soil nitrogen may, therefore, be fixed (as nitrates, etc.) (1) through the action of nitrogen-fixing bacteria in the soil (see 4, above), or (2) through the action of tubercle bacteria in root nodules. Some other groups of higher plants have tubercles with nitrogen-fixing bacteria; for example, Pavetta (Rubiaceae), with *leaf* tubercles in which atmospheric nitrogen becomes fixed.

6. [4]. **Circulation of Nitrogen in Nature.**—Free nitrogen is converted into the nitrogen of nitrates and organic nitrogen compounds by the nitrogen-fixing bacteria of the soil and by tubercle bacteria. Some free nitrogen is converted into ammonia nitrogen by the action of atmospheric electricity, the ammonia finding its way into the soil, where its nitrogen is converted into nitrite nitrogen by the nitrite bacteria. Nitrites are changed to nitrates by nitrate bacteria in the soil. Nitrates (and, to some extent, ammonium compounds and nitrites) are assimilated by higher plants and disappear in the formation of complex nitrogenous organic compounds. Animals secure their nitrogen from these complex plant compounds, or from other animals. When animal and plant tissues decay, ammonia and free nitrogen result. Free atmospheric nitrogen can be combined with other elements artifically, as in the production of calcium cyanamide ($CaCN_2$).

7. **Assimilation of Nitrogen Compounds by Lower Plants.**—Many representatives of the moulds, yeasts, and bacteria are unable to assimilate nitrates, and must be supplied with organic nitrogenous substances, or at least with ammonium salts. Animals require organic nitrogen compounds, which they secure from plants or other animals.

CHAPTER IV

ABSORPTION OF ASH-CONSTITUENTS

§1. Cultures in Artificial Media.—Besides the four elements, carbon, hydrogen, oxygen and nitrogen, every organ of the plant contains many other elements, the so-called ash-constituents. The four constituents just named volatilize and are lost during incineration, but more or less ash always remains. According to Knop, the average amount of ash left after burning plant tissue is about 5 per cent. of the original dry weight. The following elements have been found in the ash of plants:

Sulphur	Potassium	Zinc	Selenium
Phosphorus	Sodium	Mercury	Manganese
Chlorine	Lithium	Aluminium	Iron
Bromine	Rubidium	Thallium	Cobalt
Iodine	Magnesium	Titanium	Nickel
Fluorine	Calcium	Tin	Copper
Boron	Strontium	Lead	Silver
Silicon	Barium	Arsenic	

Experiments with plant cultures in artificial media show that only a few of these elements of ash are essential to normal growth. Cultures may be prepared by using either a neutral solid medium to which various salts are added, or by dissolving the respective salts in water and employing the solution thus formed. Clean quartz sand, ground pumice or ground charcoal may be used as solid media, or even finely divided platinum-wire, but the latter is very expensive. Quartz sand with various salts is most frequently used. The method of water-cultures has been well worked out in many researches dealing with the necessity of various substances for plant growth, but especially in the work of Knop and Nobbe.[1]

The study of artificially controlled cultures has shown that plants need the following elements in salts, for normal growth: nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron, and sometimes chlorine also.

These essential elements may be supplied to the plant as salts in water solution, in the following proportions by weight: one part of KNO_3, one part of KH_2PO_4, one part of $MgSO_4$, and four parts of $Ca(NO_3)_2$. A trace of ferric phosphate is also added. The addition of a nitrogen compound to the culture medium is necessary although nitrogen is not one of the ash-constituents, for plants obtain their nitrogen from the soil, as has been seen in the preceding chapter. This particular nutrient solution is known as Knop's solution. The concentration must be very low; as long as the plants are still young, 0.1 per cent.

[1] **Knop, Wilh.**, Der Kreislauf des Stoffes. Lehrbuch der Agrikulturchemie. Leipzig and St. Petersburg. 1868. P. 572–663.*

suffices, but the concentration may be raised later to 0.5 per cent.[a] The seed for the experiment may be germinated in distilled water.[b] As soon as the root has reached a suitable length, the seedling is transferred to the nutrient solution, being fixed in a perforated cork stopper with cotton packing, so that only the root reaches into the solution (Fig. 49). The culture-bottle should be protected from light, to retard or prevent the development of algæ and other organisms, and the vessel is therefore covered with a paper cylinder. Care must be taken that the culture solution does not become alkaline during the growth of the plants. To prevent alkalinity a solution of phosphoric acid may be added to the culture solution so as to make it weakly acid.[c] Normal plants, producing flowers and fruit, can be obtained in such water cultures by observing all the necessary precautions.

Salts that may be used in water-cultures are divided into two groups, those that are physiologically alkaline and those that are physiologically acid. To the first group belong salts whose anions are absorbed by the plant more

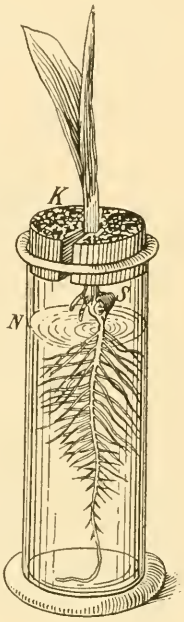


FIG. 49.—Water culture of maize seedling.

[a] This means 0.5 g. of all the salts taken together, dissolved to make 100 cc. of solution.—Various other four-salt, and some five-salt, solutions have been employed by various workers. For a list of these, see: **Grafe, Viktor,** Ernährungsphysiologisches Praktikum der höheren Pflanzen. Berlin, 1914, p, 56 *et seq.* The simplest solution yet devised for this sort of experiment is that of Shive, which contains but three salts (calcium nitrate, mono-potassium phosphate and magnesium sulphate) besides the iron phosphate. See: **Shive, J. W.,** A three-salt nutrient solution for plants. Amer. jour. bot. **2**: 157–160. 1915. **Idem,** A study of physiological balance in nutrient media. Physiol. res. **1**: 327–397. 1915.—*Ed.*

[b] Distilled water is unsuitable for seed germination and for the growth of plants, because (1) it may contain small traces of toxic substances—which are more influential in the absence of nutrient salts than in their presence—and (2) it acts to remove salts from the seeds and young seedlings by outward diffusion. See, in this connection: **True, R. H.,** and **Bartlett, H. H.,** Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. U. S. Dept. Agric., Bur. Plant Industry. Bull. 231. 1912. **True, R. H.,** Harmful action of distilled water. Amer. jour. bot. **1**: 255–273. 1914. **Merrill, M. C.,** Some relations of plants to distilled water and certain dilute toxic solutions. Ann. Missouri Bot. Gard. **2**: 459–506. 1915. **Idem,** Electrolytic determination of exosmosis from the roots of plants subjected to the action of various agents. *Ibid.* **2**: 507–572. 1915. For earlier work on the physiological properties of distilled water, see: **Livingston, B. E.,** Further studies on the properties of an unproductive soil. U. S. Dept. Agric., Bur. Soils. Bull. 36. 1907. It is probably best to allow germination to occur in a properly balanced nutrient solution, frequently renewed.—*Ed.*

[c] Frequent renewal of the solution is necessary in any case, and this avoids any need for adding acid. The salt proportions and total concentration of a nutrient solution may be maintained throughout the period of a solution-culture experiment by allowing the solution to flow continuously through the culture jar. (See: **Trelease, Sam F.,** and **Livingston, Burton E.,** Continuous renewal of nutrient solution for plants in water-cultures. Science n.s. **55**: 483–486. 1922.).—*Ed.*

rapidly than are their kations, thereby rendering the culture solution alkaline. Potassium nitrate (KNO_3) is an example of these. To the second group belong those salts whose kations are absorbed more rapidly than are the anions, thus giving the nutrient medium an acid reaction. Ammonium chloride (NH_4Cl) and ammonium sulphate [$(NH_4)_2SO_4$] are physiologically acid. The injurious effects of these salts are prevented by certain reactions in complex agricultural soils, but in sand or water cultures account must be taken of these phenomena.

§2. Importance of the Essential Ash-constituents.[1]—Not much is known concerning the importance of the single ash-constituents.[d] Of some it can be said only that their absence results in retardation of plant development. Two buckwheat plants are shown in Fig. 50, one of which has been grown in a solution containing all the essential elements and exhibits an entirely healthy appearance, while the other, cultivated in a nutrient solution lacking potassium, has hardly developed at all. The difference in growth is very great, although the dry substance of the normally grown buckwheat plant contains only about 2.5 per cent. of potassium.

FIG. 50.—Buckwheat plants in water-culture. *A*, with potassium; *B*, without potassium.

[1] **Berthelot, M.**, Chimie vegetale et agricole. Paris, 1899. Tome IV.* **Mayer, A.**, 1901–1902. [See note 1, p. 33.

[d] For modern studies on the relation between plant growth and the salt proportions and total concentration of the nutrient solution see: **Tottingham, W. E.**, A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. res. 1: 133–245. 1914. (This includes a very thorough study of Knop's solution and a review of the literature.) **Shive**, 1915, *1*, *2*. [See note *a*, p. 83.] The whole subject of the necessity of the various elements for plant growth is well discussed by **Russell**, 1915. [See note *i*, p. 73.]

The relations between plant growth and the supply of mineral salts may be studied also by using the solution-culture method and three or more *single-salt* solutions supplied separately, in rotation. This had been attempted, without success, at the Laboratory of Plant Physiology of the Johns Hopkins University, and it remained for Gericke to succeed at the University of California. (See **Gericke, W. F.**, Water culture experimentation. Science n.s. **56**: 421–422. 1922.) Gericke obtained good growth of wheat with 0.01 volume-molecular solutions for KNO_3, $CaSO_4$, and $MgHPO_4$, the solution rotation being four days for the first solution and one day for each of the other two. A very small amount of iron was supplied in the otherwise single-salt solutions. This method deserves further attention.

Sulphur is a necessary element because it is essential to the formation of proteins, which are so important in plants. It must be supplied as the sulphate of one of the essential metals; all other compounds of sulphur are injurious. It cannot be replaced by any other element.

Phosphorus also is necessary. It is a constituent of nucleins (a special group of proteins), and of phosphatides. It may be introduced in the solution only as one of the phosphates of the tribasic acid (H_3PO_4), since other phosphorus compounds have been found to be harmful. It cannot be replaced by any other element.

Potassium is also absolutely essential. It accompanies carbohydrates and is supposed to promote their formation.

Calcium is likewise necessary, especially for normal leaf development. Some plants without chlorophyll (moulds) can exist without calcium,[1] and non-green phanerogams contain much less calcium than do green plants.[2]

Magnesium is also necessary; it accompanies proteins and is contained in chlorophyll.

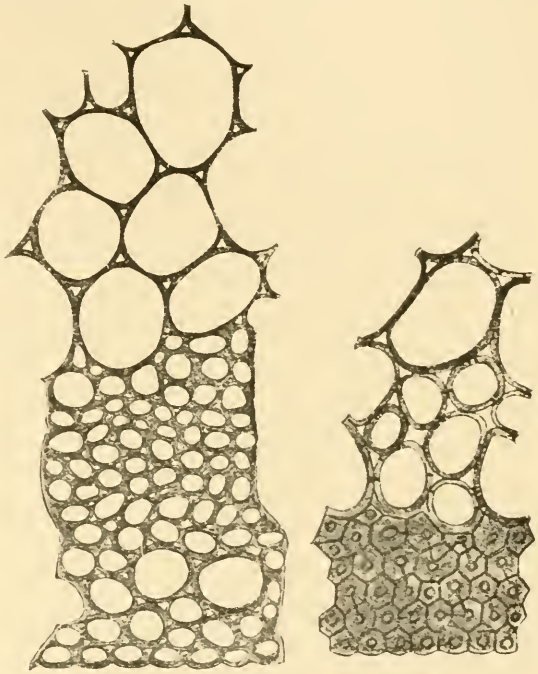


FIG. 51.—Portion of a cross-section through a rye stalk. At left, lodged; at right, normal. (*After Koch.*)

Finally, plants need *iron*, the lack of which prevents chlorophyll formation; they become pale and chlorotic,[3] even in the light, when grown without this element.

§3. **Importance of the Non-essential Ash-constituents.**—Plant ash contains appreciable quantities of other elements than the absolutely essential ones, and these are not to be considered as entirely without physiological effects. Each ash-constituent must be considered as exerting some slight effect in the plant, either injurious or beneficial. If plants develop apparently normally in a nutrient solution without a given element, it does not necessarily follow that this element, if present might not exert some beneficial influence.

Silicon, for example, is abundant in many plants. Nevertheless, experiments with various plants in artificial media have shown that even the grasses

[1] **Loew, Oscar,** Liming of soils from a physiological standpoint. U. S. Dept. Agric. Bull. 1. 27 p. Washington, 1901.

[2] **Aso, K.,** On the lime content of phanerogamic parasites. Bull. Coll. Agric. Imp. Univ. Tokyo 4: 387–389. 1900–1902.

[3] **Molisch,** 1892. [See note *b*, p. 51.]

(Gramineæ) can develop without this element. The lodging of grain (when the plants fail to stand erect in the field), which was earlier ascribed to a deficiency of silicic acid (H_2SiO_3) in the soil, is a result of insufficient illumination. This, in turn, is due to too thick planting. Anatomical study[1] of the stalks of lodged grain shows that they have all the characteristics of etiolated stems (Fig. 51). In healthy stems we find small, thick-walled cells, while in etiolated stalks, whether lodged or not, the cells are very large and have much thinner walls.

In laboratory experiments, where plants are protected from some of the unfavorable conditions of the field, silicon is not essential, but this is not true when plants develop under natural conditions. Here silicon appears to play a very important rôle, protecting the plant from attacks of various parasites. Fungus hyphæ cannot easily penetrate cell walls that are impregnated with silica. Wheat, rye, etc., grown in nutrient solutions deficient in silicic acid often suffer so severely from rust that only great care can prevent their complete destruction. The hardness of silicated cell walls is also a very good protection against animal attack. Thus, for instance, one plant of *Lithospermum arvense*, grown in a nutrient solution without silicic acid, suffered severely from plant-lice even though these were removed daily, while two similar plants, standing near by and grown in similar solutions but not tended so carefully, were completely killed by these insects.

The distribution of silicic acid in different parts of seeds[2] is another indication of its protective action. Millet seeds without the seed-coats contain only from 4.8 to 7.1 per cent. of the total silicic acid of the seed, all the remainder (from 92.6 to 95.1 per cent.) being deposited in the seed-coats. Such a marked accumulation of silicic acid in the seed-coats suggests the importance of this substance to plants growing under natural conditions. The investigations of Sabanin upon ripening seeds of millet show that this plant hastens, as it were, to accumulate enough silicic acid in the peripheral parts of the grain (as in the palea) to protect the increasing reserve material from unfavorable external conditions.

Most plants can live without chlorine, but buckwheat deprived of this element did not attain complete development in Nobbe's experiments, and it was his opinion that chlorine favors the translocation of carbohydrates from the leaves into other organs. Knop, however, obtained normal development of buckwheat plants in a solution without chlorine, and so the question of the rôle of chlorine is still unsettled.[e] It is advisable to add chlorine to the nutrient

[1] **Koch, L.**, Welche abnorme Aenderungen werden durch Beschattung in wachsenden Pflanzenorganen hervorgerufen? Landw. Centralbl. Deutschl. **20**: 202. 1872.

[2] **Sabanin, A. N.**, Ueber Kieselsäure in den Körnern der Hirse (*Panicum miliaceum L.*) [Abstract in German, pp. 295–302. Text in Russian.] Jour. exp. Landw. **2**: 257–302. 1901.

[e] Buckwheat has been repeatedly grown to maturity, with production of seed, in water-cultures without any more chlorine than might have been present in spite of all ordinary precautions to exclude this element, in the Laboratory of Plant Physiology of the Johns Hopkins University; but the possibility remains that the presence of chlorine might produce more vigorous growth. Trelease's results strengthen the idea that this element is not beneficial to wheat in its early stages of growth. It exerted no injurious influence, however, in his cultures. (See: **Trelease, Sam F.**, The relation of salt proportions and concentrations to the growth of young wheat plants in nutrient solutions containing a chloride. Philippine jour. sci. **17**: 527–603. 1920.).—*Ed.*

solution when experimenting with plants whose relation to chlorine is not understood; potassium chloride is best for this purpose. Observations of agriculturists favor the idea that chlorine influences the translocation of carbohydrates under natural conditions. Potatoes grown in soil rich in chlorine contain less starch than those cultivated in soil deficient in this element. So, when potatoes with the highest possible starch content are desired chlorine fertilizers are to be avoided.[1]

Zinc is one of the less common ash-constituents. It is contained in a variety of violet (*Viola calaminaria* or *V. lutea* var. *multicaulis*), which grows exclusively in soils containing zinc. The differences by which these "calamin" violets are distinguished from the ordinary *Viola tricolor* are probably due to the effect of the zinc salt.[f] Also, Raulin used zinc in his nutrient solution (see page 46) for *Aspergillus niger*. Rikhter's[2] investigations showed that zinc promoted growth and the accumulation of organic substances during the early period of development of this mould, but prevented the formation of spores. Kostychev[3] also found that zinc influenced metabolism in moulds.

Aluminium occurs in plant ash rather infrequently. It influences the color of the flowers in Hydrangea (*H. hortensis*).[4] Gardeners had long since noticed that the ordinary reddish-flowered hydrangea bore blue flowers when grown in certain soils, such as some forest and moor soils. Tests of many different substances showed that blue flowers always appeared if the soil contained soluble aluminium compounds. At first ordinary alum (made up of aluminium and potassium sulphate, $Al_2SO_4 + K_2SO_4 + 24H_2O$) was used, being introduced into the soil in pieces varying from the size of a pea to that of a hazel-nut, and blue flowers were always obtained. In another series of experiments, some plants were treated with aluminium sulphate and others with potassium sulphate. The cultures with potassium sulphate gave the usual red color, while those with aluminium sulphate always produced blue flowers, and the color appearing with this salt was more intense than that obtained by the alum treatment. The alum therefore produced the blue color because of the presence of aluminium, the potassium being without influence. This case shows clearly how the presence of a non-essential element may influence metabolism in a specific manner.

Researches in recent years have shown that various elements, such as manganese, boron, rubidium, etc., are more or less favorable to plant growth.

[1] **Budrin,** Die künstlichen Düngemittel mit besonderer Berücksichtigung der Stickstoffdünger. Warsaw, 1888. (Russian.)* [See also: **Tottingham, Wm. E.,** A preliminary study of the influence of chlorides upon the growth of certain agricultural plants. Jour. Amer. Soc. Agron. **11**: 1–32. 1919.]

[2] **Richter, Andreas,** Zur Frage der chemischen Reizmittel. Die Rolle des Zn and Cu bei der Ernährung von Aspergillus niger. Centralbl. Bakt. *II*, **7**: 417–429. 1901.

[3] **Kostytschew, S.,** Der Einfluss des Substrates auf die anaërobe Athmung der Schimmelpilze. Ber. Deutsch. Bot. Ges. **20**: 327–334. 1902.

[4] **Molisch, Hans,** Der Einfluss des Bodens auf die Blüthenfarbe der Hortensien. Bot. Zeitg. **55**: 49–61. 1897.

[f] But the studies of Hoffmann appear to controvert this statement. According to this author the calamin violet is the same whether grown with or without zinc, and *Viola tricolor* does not take the calamin form when supplied with zinc. See: **Hoffmann, H.,** Culturversuche. Bot. Zeitg. **33**: 601–605, 617–628. 1875. **Idem,** Untersuchungen über Variation. Ber. Oberhess. Ges. Giessen **16**: 1–37. 1877.—*Ed.*

These elements act like catalyzers,[1] while the plastic ash-constituents (phosphorus, sulphur, potassium, magnesium, calcium) have to do with the structure of the cell and its parts; these latter may also act as catalyzers, however.

§4. **Ash-analysis of Plants.**—Besides the growing of plants in artificial media, the analysis of plants grown under natural conditions is also useful in the determination of the relative importance of the various mineral elements. Large numbers of such analysis have been carried out, and the results obtained up to 1880 have been assembled and arranged in a very helpful way by Wolff.[2]

The ash-analyses of entire plants show that the amount of each individual ash-constituent is different with different plants. The agriculturist, for example, recognizes three groups of cultivated forms, silicon plants, calcium plants and potassium plants, according to which one of these three elements is most abundant in the ash. The following table (after Liebig) contains the results of ash-analyses of some of the plants belonging to these three classes.

		Salts of K and Na	Salts of Ca and Mg	Silicic Acid
		per cent.	*per cent.*	*per cent.*
Silicon plants	Oat straw and grain...	34.00	4.00	62.08
	Rye straw.....	18.65	16.52	63.89
Calcium plants	Havanna tobacco.....	24.34	67.44	8.30
	Stems and leaves of pea	27.82	63.74	7.81
Potassium plants	Sugar cane.....	88.80	12.00	.....
	Artichoke	84.30	15.70	.....

The total amount of ash is also known to be different in different species. Water plants are richest, woody plants are among the poorest, and herbs take a middle place, with reference to the amount of ash they contain. A comparison of the ash-analyses of the alga Chara and the tree Fagus (beech) is shown in the next table.

	Entire Ash Content, Per Cent. of Dry Weight	Amounts of Various Elements in Ash Calculated as Oxides, Per Cent. of Total Ash						
		K_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2
Chara fœtida.....	39.080	0.40	96.23	1.39	0.28	0.28	0.49	0.58
Fagus sylvatica								
Wood	0.355	14.40	60.20	4.50	2.30	2.70	3.50	10.00
Bark.....	5.860	5.10	83.40	3.60	0.70	2.10	1.00	3.70
Leaves.....	5.140	21.80	44.30	7.20	2.30	7.80	2.40	10.50

[1] **Agulhon, H.,** Recherches sur la présence et le rôle du bore chez les végétaux. Paris, 1910.

[2] **Wolff, Emil,** Aschen-Analysen von landwirthshaftlichen Producten, Fabrik-abfallen und wildwachsenden Pflanzen. I Theil. Berlin, 1871. **Idem,** Aschen-Analysen von land- and forstwirtschaftlichen Producten II Theil. Berlin, 1880.

This distribution of the ash shows that the tissues richest in ash are those in which living cells are most numerous, such as those of algæ and the leaves and cortex of the beeech. Dead cells contain much less ash, since the salts begin to pass out at about the time death occurs; thus, the hard wood of the beech contains much less than does the dry substance of the living leaf tissue.

Different amounts of ash occur in different organs of the same plant. Leaves are richer in ash than stems and roots. The amounts of the different chemical elements likewise vary; calcium, for instance, predominates in leaves.

The ash content of each organ changes during the course of its development; in leaves it increases with age, while in roots and stems it decreases. In the case of roots and stems the number of dead cells, poor in ash, increases with age. The following table gives the total ash content and the proportions of the various elements in the ash, for beech leaves (*Fagus sylvatica*) at three different stages of their development.

Date	Total Ash, Per Cent. of Dry Weight	Amounts of Various Elements in Ash, Calculated as Oxides, Per Cent. of Total Ash					
		K_2O	CaO	MgO	Fe_2O_3	P_2O_5	SiO_2
May 16	4.1	42.1	13.8	4.3	0.8	32.4	1.6
July 18	4.7	17.1	42.3	5.6	1.4	8.2	21.3
Oct. 15	7.1	7.1	50.6	4.1	1.3	5.1	30.5

These analyses of beech leaves show how strikingly the amount of the different ash-constituents alter with the age of the leaves. Calcium and silicon show a marked increase in amount while potassium and phosphorus decrease as the leaves become older. But, as has been well pointed out by Wehmer,[1] it is not to be concluded from these analyses that the absolute amounts of potassium and phosphoric acid diminish in such leaves. For example, if 50 g. of potassium and 50 g. of other elements were present in a certain quantity of young leaves, we should then find 50 per cent. of potassium in the ash. If we suppose that the leaves take up 100 g. more of the other elements but that the amount of potassium remains unchanged, then we should expect to find only 25 per cent. of potassium in the ash of the older leaves. According to Riesmüller's analyses, the ash of 1000 beech leaves contained, at different times of the year, the percentages and absolute amounts of potassium shown in the following table.

[1] **Wehmer, C.**, Zur Frage nach der Entleerung absterbender Organe, insbesondere der Laubblätter. Unter Berüchsichtigung der vorliegenden Aschenanalysen vom kritischen Standpunkte beleuchtet. Landw. Jahrb. 21: 513–569. 1892.

Time of Analysis	Total Ash Content	Absolute Amount of Potassium
	per cent.	*grams*
May	31.2	0.7
June	21.7	1.2
July	11.8	1.2
August	9.8	1.1
October	7.6	0.8
November	5.7	0.7

The percentage content of potassium in the ash underwent a marked decrease during the course of the summer, but no corresponding decrease in the absolute amount of potassium is apparent. The absolute amount is maintained fairly constant during the growing period, and undergoes a marked decrease only in late autumn. Similar results were also obtained for phosphoric acid (PO_4).

§5. **Microchemical Ash-analysis.**[g]—Ash-analyses of the kind just referred to can be carried out only with large amounts of material, but in exact studies of the distribution and translocation of ash-constituents small quantities must suffice, and microchemical analysis is resorted to in such cases.[1] Platinic chloride is used for the identification of potassium, beautiful crystals of potassium chloroplatinate being formed (Fig. 52). To identify calcium, dilute sul-

[1] **Haushofer, K.,** Mikroskopische Reaktionen. Braunschweig, 1885. **Klément, Constantin,** and **Renard, A.,** Réactions microchimiques à cristaux et leur application en analyse qualitative. 132 p. Bruxelles, 1886. **Schimper, A. F. W.,** Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**: 207–261. 1890. P. 207. [**Zimmerman, A.,** Die botanischen Mikrotechnik. Tübingen, 1892. **Idem.** Botanical microtechnique, a handbook of methods for the preparation, staining, and microscopical investigation of vegetable structures. Translated by J. E. Humphrey. XII + 296 p. New York, 1893. **Richter, O.,** Die Fortschritte der botanischen Mikrochemie seit Zimmermann's *Botanische Mikrotechnik.* Sammelreferat Zeitsch wiss. Mikroskopie **22**: 1904–261. 1905. **Emich, F.,** Lehrbuch der Mikrochemie. Wiesbaden, 1911. **Molisch, Hans,** Mikrochemie der Pflanze. Jena, 1913.]

[g] On these methods for ash-analysis the reader is referred to Molisch, 1913, cited just below. The following points may be of value in connection with the discussion given in the text. The reaction given for *potassium* fails to distinguish between potassium and ammonium. (On this difficulty see: **Weevers, Th. I.,** Untersuchungen über die Lokalization und Funktion des Kaliums in der Pflanze. Recueil trav. bot. néerland. **8**: 289. 1911.) When *calcium* is plentiful the crystals mentioned occur in dense masses, so that their individual form is seen only at the periphery of the mass. The reaction here given for *iron* serves only to identify it when in the ferrous condition. For other tests for this element in inorganic compounds see Molisch, 1913. In organic compounds (*masked* iron) it cannot be identified by any known microchemical methods. (See: **Wiener, Adele,** Microchemical proof of iron, especially masked, in plants. Rev. in: Chem. abstracts **11**: 615–616. 1917. [Original not seen; cited as: Biochem. Zeitsch. **77**: 27–50. 1916].) To identify *phosphorus* in organic compounds it is necessary first to incinerate the material, after which the test given may be applied. The precipitation of the phosphate ion as ammonium-magnesium phosphate (see under magnesium) offers a more sensitive method, not affected by the presence of organic substances. (See Molisch, 1913.) The tests for *sulphur* given in the text apply only to sulphates and are, moreover, not reliable for plant tissues. There is no microchemical test available for sulphur as it is usually encountered in plant cells. A more reliable test for *chlorides* is that of Macallum. (See: **Macallum, A. B.,** On the nature of the silver reaction in animal and vegetable tissues. Proc. Roy. Soc., London **B 76**: 217–229. 1905.)—*Ed.*

phuric acid is added, which forms needle-like crystals of calcium sulphate (gypsum) in the presence of this element (Fig. 53). Magnesium crystallizes, as ammonium-magnesium phosphate (in a great variety of forms), upon the

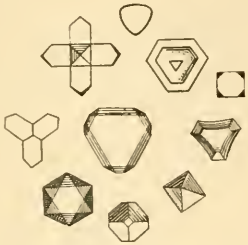


Fig. 52.—Crystals of potassium chloroplatinate.

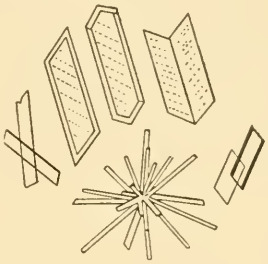


Fig. 53.—Crystals of calcium sulphate.

addition of sodium phosphate and ammonia (Fig. 54). Iron is identified by the blue color produced with potassium ferrocyanide. Phosphates are identified by treatment with a solution of ammonium molybdate in nitric acid, greenish-

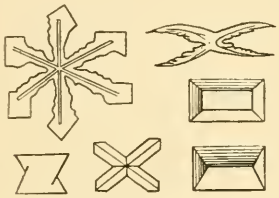


Fig. 54.—Crystals of ammonium magnesium phosphate.

Fig. 55.—Crystals of ammonium phospho-molybdate.

yellow crystals of ammonium phospho-molybdate being formed and gradually becoming bright green (Fig. 55). Upon addition of strontium nitrate, sulphur separates out as small rounded crystals of strontium sulphate (Fig. 56). An-

Fig. 56.—Crystals of strontium sulphate.

Fig. 57.—Crystals of thallium chloride.

other test for sulphuric acid is the addition of cæsium chloride and aluminium chloride, which leads to the formation of large crystals of cæsium-alum. Chlorides may be identified by adding thallium sulphate, with the formation of characteristic crystals of thallium chloride (Fig. 57).

§6. The Plant and the Soil.[h]—Plants obtain all their essential ash-constituents from the soil. The following table gives an idea of the compositions of several different kinds of soil, the numbers representing the amounts of usual soil bases, calculated as oxides and expressed as percentages of the total dry weight of the soil.

	Loam	Loamy Marl	Lime Marl
SiO_2	51.52	40.70	11.80
Al_2O_3	17.93	32.00	10.60
Fe_2O_3	7.42	8.90	1.50
CaO	1.57	6.00	47.00
MgO	7.27	1.20	0.20
K_2O	4.10	0.05	0.10

Every soil covered with vegetation contains organic as well as mineral substances. Bog soils are particularly rich in organic materials, as is evident from the following table, which again presents percentages on the basis of the dry weight of the soil.

	P_2O_5	N	Humus
Black soil, Government of Orlov, Russia	0.128	0.268	13.080
Black soil, Government of Saratov, Russia	0.223	0.607	14.580
Soil of low moor	0.250	3.230	82.560
Soil of high moor	0.090	1.060	91.470

The chemical analysis of a soil can give no definite idea of its properties, however.[i] In order to predict a good crop from a given soil, it is not enough to know that it contains potassium, phosphorus and the other essential elements; it must also be known whether these elements occur in compounds that plants can assimilate. Nile silt, famous for its fertility, contains only 0.5 per cent. of potassium and needs no further addition of this element, but mica-schist soil contains 3 per cent. of potassium and remains unproductive unless a potassium fertilizer is added.

To obtain a better idea of the productiveness of a soil, the analysis of its water or hydrochloric acid extract is carried out, in addition to determining the essential minerals present. The necessary elements for plant growth are contained in very small quantities in the extract, but it must be borne in mind that

[h] An excellent treatise on the soil is: **Mitscherlich, E. A.**, Bodenkunde für Land- und Forstwirte. 2 Aufl. 317 p. Berlin, 1913. A less scientific treatise is: **Hilgard, E. W.**, Soils, their formation, properties, composition, and relations to climate and plant growth in the humid and arid regions. 593 p. New York, 1912. Best of all presentations of the soil, from the standpoint of plant physiology, is that of **Russell** (1921). [See note *i*, p. 73].—*Ed.*

[i] **Cameron, F. K.**, The soil solution. 136 p. Easton, Pa. 1911.—*Ed.*

not nearly all of the materials thus extracted from the soil can be assimilated by the plant, and also that much material that the plant might eventually absorb is not thus extracted. It must also be emphasized that plant species differ very greatly in their power to absorb salts from the soil.

If the soil does not contain the essential elements in a sufficient amount and in the proper form for assimilation by plants, its productiveness may be increased by the addition of suitable fertilizers. The gain that may be obtained from the use of the fertilizer depends not only upon the properties of the latter but also upon those of the soil and upon the plant species that is to be culti-

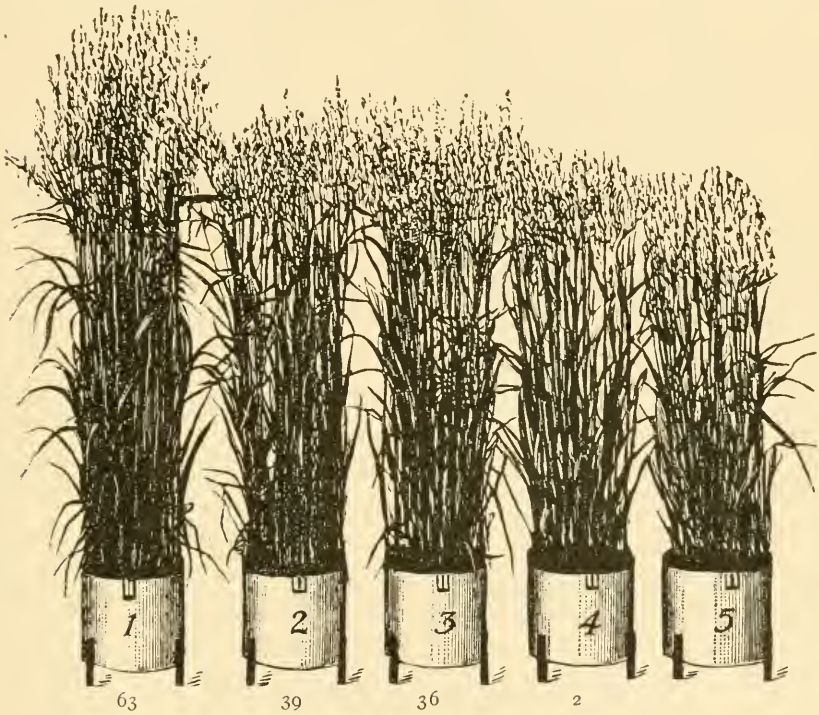


FIG. 58.—Effect of fertilizing oats with different kinds of Thomas slag (1–3) and with phosphorite (4), all showing different solubilities of their phosphates in ammonium citrate solution. The relative solubilities of the phosphates are shown by the numbers below the pots. Culture 5 received no addition. (*After P. Wagner.*)

vated. For example, let us consider phosphatic fertilizers. Thomas slag is one of the best of these. It is a by-product derived from the manufacture of steel out of pig iron. The latter contains silicic acid, sulphur and phosphorus, which are oxidized, through the addition of lime in the process, to calcium salts, and these rise to the surface of the molten steel as slag. Such slag varies according to the solubility of its phosphoric acid in an acid solution of ammonium citrate. The varieties with large amounts of phosphates that are soluble in ammonium citrate are good fertilizers, while other varieties are not useful in this way.

This is shown by Wagner's experiments[1] with oats (Fig. 58). Three culture vessels received equal amounts of phosphoric acid (0.5 g.) as pulverized Thomas slag; but different kinds of slag were used, showing different solubilities of their phosphates in ammonium citrate solution. The fourth vessel received twice as much phosphoric acid (1.0 g.), in the form of pulverized phosphate rock (phosphorite), and the fifth received no phosphorus fertilizer at all. The following table shows the effects of these fertilizers upon the growth of the plants.

Culture No.	Phosphoric Acid Added	Kind of Fertilizer	Solubility in Ammonium Citrate	Yield	Gain Due to Fertilizer
	grams		*per cent.*	*grams*	*per cent.*
1	0.5	Thomas slag.....	65	416.7	272.7
2	0.5	Thomas slag.....	39	306.9	162.9
3	0.5	Thomas slag.....	36	281.1	137.1
4	1.0	Phosphorite.....	2	159.0	15.0
5	.....	No fertilizer.....	....	144.0	..

This experiment shows very clearly how fertilizers may differ in quality. Although the fourth culture contained more phosphoric acid than any of the others, its yield exceeded that of the unfertilized plants by only about 15 g.; the plants could not assimilate this particular phosphorus compound. It appears that the greater the amount of phosphorus compounds that can be dissolved out of the fertilizer by ammonium citrate solution, the better can the fertilizer be utilized by the plant and the greater is the yield.

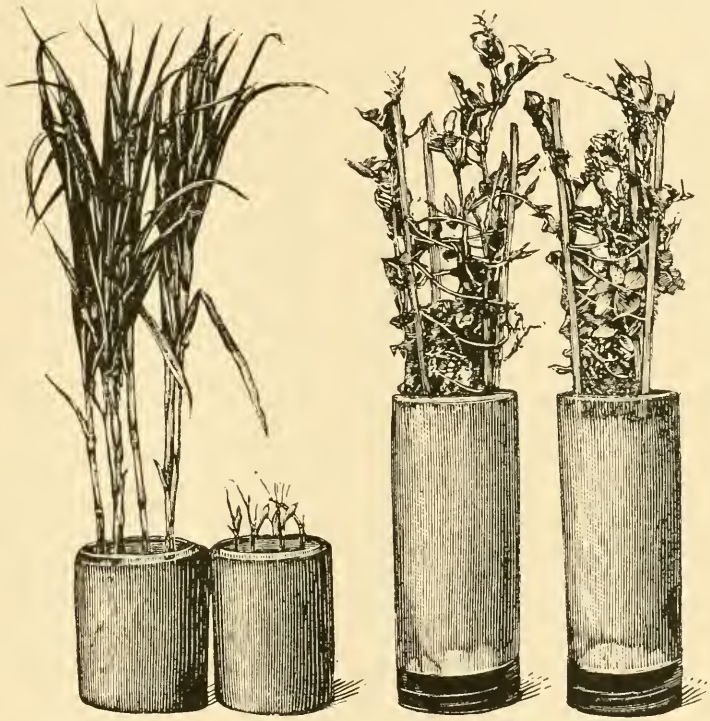
Not only the properties of the fertilizer, but also the peculiarities of the plants under cultivation must receive attention. The same fertilizer, added to a given soil, may be beneficial to one plant and entirely useless to another. In Prianishnikov's experiments,[2] for instance, various plants were cultivated in sand supplied with the necessary nutrient salts. In one series of experiments phosphorus was supplied as mono-sodium phosphate (NaH_2PO_4), in the other as phosphate rock (phosphorite), which contains calcium phosphate, calcium carbonate, sand, loam, iron oxide, and aluminium. Millet grown in these two media gave a yield of 29.07 g. with the soluble phosphate and one of only 0.57 g. with phosphate rock (Fig. 59). Millet and other grains either cannot utilize phosphorite in sand cultures at all, or else they can utilize it only to a very slight degree. The Papilionaceæ (peas, beans, etc.), however, show an entirely different behavior toward phosphate fertilizers. Scarcely any difference can be discovered between pea cultures supplied with soluble phosphates and those supplied with phosphorite (Fig. 59).

The value of phosphate rock as a fertilizer depends not only upon the nature

[1] **Wagner, Paul,** Düngungsfragen unter Berücksichtigung neuer Forschungsergebnisse. Heft III. 56 p. Berlin, 1896.

[2] **Prianishnikov, D. N.,** Ist die Phosphorsäure der Mineralphosphate der Kulturpflanzen zugänglich? [Russian, with German abstract.] Ann. Inst. Agron. Moscou 5 (Partie non officielle): 90–110. 1899.

of the plant but also upon that of the soil. That the small grains fail to assimilate phosphorite in sand cultures does not necessarily mean that they behave in the same way in cultures with other kinds of soil. In Prianishnikov's experiments summer-rye was grown in black soil from the Government of Voronezh, in light sandy loam from the Government of Minsk, and in two light-colored, uncultivated sands ("Podsol") from the vicinity of Moscow, all four soils being fertilized with phosphate rock. His results are presented in the following table.



NaH_2PO_4 Phosphorite NaH_2PO_4 Phosphorite

FIG. 59.—Comparative effects of sodium phosphate and of phosphorite upon millet and pea in sand cultures. (*After Prianishnikov.*)

Soil	Yield of Grain		Total Weight of Grain and Straw		Increase in Yield Due to Fertilizer
	Unfertilized	Fertilized with Phosphorite	Unfertilized	Fertilized with Phosphorite	
	grams	*grams*	*grams*	*grams*	*per cent.*
Black soil	1.95	2.30	5.65	5.80	3
Sandy loam	1.25	1.50	3.55	4.40	24
Sand No. 1	0.40	4.75	3.30	10.75	226
Sand No. 2	1.40	3.30	2.35	11.10	372

Phosphorite fertilizers had very good effects upon the uncultivated sands (Podsol), but no effect at all upon the black soil. The sands apparently increased the solubility of phosphate rock, since summer-rye cannot assimilate phosphoric acid in the form in which it occurs in this fertilizer, and the black soil appears to have had no such effect.

In sand cultures phosphorite can be made available for the small grains by supplying them with a complementary fertilizer, such as ammonium salts, which are physiologically acid. Since adequate amounts of ammonium salts are usually injurious to plants in water and sand cultures, Prianishnikov[1] replaced only a part of the requisite sodium nitrate in his sand cultures by an equivalent amount of ammonium sulphate. This gives a medium that tends

Fig. 60.—Effect of ammonium salts upon the availability of phosphorite for oats in sand cultures. (*After Prianishnikov.*) See text for explanation.

to become more acid with increase in its content of the ammonium salt, and so phosphate rock supplied to such cultures might be expected to become soluble and thus available to the plants. This expectation was realized in experiments with oats. The results of such an experiment are given in the table below. The appearance of the first six cultures, in the order followed in the table, is shown in Fig. 60.

Culture No.	Treatment	Weight of Tops *grams*
1	Control, $KH_2PO_4 + NaNO_3$	19.7
2	Phosphorite $+ NaNO_3$	6.9
3	Phosphorite $+ \frac{1}{4}(NH_4)_2SO_4 + \frac{3}{4}NaNO_3$	22.0
4	Phosphorite $+ \frac{1}{2}(NH_4)_2SO_4 + \frac{1}{2}NaNO_3$	20.5
5	Phosphorite $+ \frac{3}{4}(NH_4)_2SO_4 + \frac{1}{4}NaNO_3$	19.2
6	Phosphorite $+ (NH_4)_2SO_4$	1.6

[1] Prianishnikov, D. N., Results of vegetation experiments for 1899 and 1900. [Russian.] Bull. Moscow Agric. Inst. 7 (non-official part): 85–129. 1901.

These results support the idea that partial replacement of sodium nitrate by ammonium salts renders the phosphoric acid of the phosphate rock available for oats; when one-fourth or one-half of the $NaNO_3$ was replaced by $(NH_4)_2SO_4$ the yield did not fall below that of the control, as it did in the other cases.

It is clear that the nutrient materials in the soil are utilized to unequal degrees by different plants. As we shall see later, roots excrete acid substances that favor the solution of soil materials otherwise practically insoluble in water. Furthermore, many plants are characterized by having their roots covered with fungus hyphæ, a fact discovered by Kamienski.[1] Frank[2] gave the name *mycorhiza* to this weft of fungus hyphæ growing upon roots, and emphasized the importance of this whole phenomenon in the physiology of nutrition. Plants that have mycorhiza are said to be *mycotrophic.* We owe extended investigations upon the physiological importance of mycorhiza to Stahl.[3] In some cases the fungus hyphæ cover the surface of the roots (ectotrophic mycorhiza), as is shown in the case of beech roots (Fig. 61). The tip region of

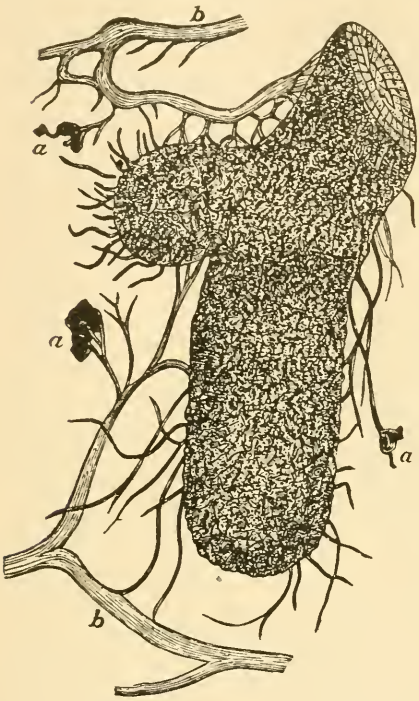


FIG. 61.—Ectotrophic mycorhiza of the beech; *a*, humus particles; *b*, strands of fungus hyphæ penetrating the soil.

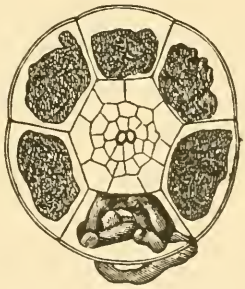


FIG. 62.—Endotrophic mycorhiza in epidermal cells of the root of *Andromeda polifolia*, the root shown in cross-section.

the root is covered with hyphæ some of which branch out into the soil and attach themselves to particles of humus. In other cases the fungus hyphæ are found within the cells of the root (endotrophic mycorhiza), as in the case of *Andromeda polifolia* (Fig. 62). Here the hypæ occur in the large cells of the root epidermis.

Mycorhiza is of common occurrence, being found on the majority of vascular plants, not only trees, shrubs and herbs, but even mosses. Some plants cannot

[1] **Kamienski, Fr.,** Die Vegetationsorgane der Monotropa hypopitys L. Vorläuf. Mitth. Bot. Zeitg. 39: 457–461. 1881.

[2] **Frank, B.,** Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. Deutsch. Bot. Ges. 3: 128–145. 1885.

[3] **Stahl, E.,** Der Sinn der Mycorhizenbildung. Ein vergleichend-biologische Studie. Jahrb. wiss. Bot. 34: 539–668. 1900.

thrive without mycorhiza, others are never found with it, and still others occur sometimes with and sometimes without. The non-green seed-plants appear generally to belong to the first group. Mycorhiza develops mainly in soils rich in humus, where the fungus hyphæ facilitate the entrance of nutrient substances into the plant.

Non-green seed-plants draw organic as well as inorganic substances from the soil by means of their mycorhiza. The importance of mycorhiza to green plants is probably most pronounced in connection with the absorption of the ash-constituents, although these may be taken up first in organic compounds. The properties of humus soils are not by any means to be considered only from a purely chemical standpoint. The abundance of bacterial and fungous organisms in the soil makes it almost like a living thing, and all the microörganisms of the soil require large amounts of mineral substances. If a higher green plant grows in humus soil it must compete with these microörganisms for its nutrition, and this competition is especially active since the nutrient materials in humus are not as well suited to the needs of green plants as are those in mineral soils.

FIG. 63.—Cultures of *Lepidium sativum* in humus soil. On the left, two vessels with sterilized soil; on the right, two vessels with unsterilized soil. (*After Stahl.*)

It appears that plants with an associated fungus, forming mycorhiza, are thus enabled to compete with soil microörganisms not associated with them much more successfully than can plants without mycorhiza. How difficult the growth of these latter may be in humus soil is shown by the following experiment of Stahl. Humus soil from a beech forest was placed in four vessels, two of which were sterilized with ether and chloroform vapor, thus killing all the microörganisms of the soil without otherwise altering it. Seeds of *Lepidium sativum*, a plant without mycorhiza, were then planted in all four vessels. Healthy plants developed in the sterilized vessels, while the plants grew but poorly in those that were not sterilized (Fig. 63). The microörganisms of the soil are thus seen to have retarded the growth of Lepidium to a very marked degree.

No trace of nitric acid or nitrates can be found in the mycorhiza, nor is any usually found in soils in which mycotrophic plants are growing. This fact confirms the opinion that mycotrophic plants differ from those without mycorhiza in their manner of nutrition. If fact, the experiment with ammonium fertilizers, mentioned above, shows that such fertilizers have no effect in soils rich in humus and poor in lime (which are usually occupied by mycotrophic plants), and that intrification progresses with great difficulty in these soils.

If a particular kind of plant is grown for several years in succession upon the same soil, the crop gradually decreases, in spite of the addition of plenty of fertilizers. This is the well-known phenomenon of "soil sickness." In this case we do not have to deal with an inadequate supply of mineral nutrients, but with something entirely different. The work of Whitney and Cameron, and that of Livingston, Schreiner, and other American investigators,[1] has indicated that plants produce poisonous substances (toxins) in the soil.[i] These toxins appear, in many cases, to be poisonous only to the particular kind of plant in connection with which they were produced, and this may explain the fact that

[1] **Whitney, Milton,** and **Cameron, F. K.,** Investigations in soil fertility. U. S., Dept. Agric. Bur. Soils. Bull. 23. 48 p. Washington, 1904. **Livingston, B. E., Britten J. C.,** and **Reid, F. R.,** Studies on the properties of an unproductive soil. *Ibid.* Bull. 28. 39 p. Washington, 1905. **Livingston,** 1907. [See note *b*, p. 83.] **Schreiner, Oswald, Reed, Howard S.,** and **Skinner, J. J.,** Certain organic constituents of soils in relation to soil fertility. *Ibid.* Bull. 47. 52 p. Washington, 1907. **Schreiner, Oswald,** and **Shorey, Edmond C.,** The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc. 30: 1295–1307. 1908. **Idem,** The isolation of dihydroxy-stearic acid from soils. *Ibid.* 30: 1599–1607. 1908. **Idem,** The isolation of harmful organic substances from soils. U. S. Dept. Agric., Bur. Soils. Bull. 53. 53 p. Washington, 1909.

[i] A discussion of some of the earlier literature regarding this general idea of soil toxins is given by **Livingston,** 1907. [See note *b*, p. 83.] This earlier literature (not considered by **Whitney** and **Cameron,** 1904, nor by **Livingston** *et al.*, 1905 [note 1, just above]) is rather extensive. The idea that plants may excrete into the soil substances that may be poisonous to other plants, appears to have originated with **A. P. DeCandolle** (Physiologie végétale. Paris, 1832), but the experimentation invoked by this writer's suggestion seemed to disprove the hypothesis, and the whole matter was laid aside until it was taken up again, in a modern way, by the Duke of Bedford and S. U. Pickering (at the Woburn Experimental Fruit Farm, near Bedford, England) and by the American students mentioned above. On the Woburn work see: **Pickering, Spencer U.,** The effect of grass on apple trees. Jour. Roy. Agric. Soc. England **64** (of entire series): 365–376. London, 1903. Also see the Reports of the Woburn Experimental Fruit Farm after 1897.

In later years the general hypothesis that unproductiveness in agricultural soils is *frequently* due to soil toxins has been well established by workers in various parts of the world, and it is now generally accepted. Evidence that agricultural plants do actually *excrete* toxic substances into the soil is not very strong in any of this work, however. Better than to assert that they are so excreted is to state that there is evidence that the soil frequently *contains* toxins and that these sometimes result, directly or indirectly, from the growth of higher plants. As to the manner in which these poison substances arise in the soil, no definite statements can yet be made, but they are surely not generally excreted *as such* from plant roots. There is physiological evidence, however, that such substances are given off by living roots when the latter are practically deprived of oxygen. (See p. 126.) It seems highly probable that soil microörganisms play an important part in the production of the toxic substances here considered. Excreted substances, the materials of dead root-cap cells, root-hairs, roots, etc., or even substances carried down into the soil by rain (as from the bark of trees and fallen leaves) may become altered by the action of microörganisms so as to produce poisons. That such poisons *are present* in many soils has now been established without question by Schreiner and his coworkers, and also that their deleterious effect upon plants may often be removed by oxidation, or by the addition of proper substances.

The general acceptance of the hypothesis of toxic soil constituents as a frequent cause of unproductiveness was much retarded by the form of its original statement, by Whitney and Cameron (1904), which emphasized actual root excretion at the expense of all the other logical possibilities. It was of course to be expected that such poisons might arise in the soil in a great variety of ways, and the theory of soil toxins is not to be considered without continual reference to the microbiology of the soil. **Russell** (1921. [see note *i*, p. 73.]) gives a clear discussion of this whole matter, from the standpoint of field experiments.—*Ed.*

FIG. 64.—Wheat plants grown in extract of toxic soil. 1 and 2, undiluted extract; 3 and 4, equal parts of extract and distilled water; 5 and 6, one part extract diluted with nine parts of distilled water. (*After Schreiner and Shorey. Reproduced by permission of U. S. Dept. Agric.*, 1909.)

FIG. 65.—Vicia faba (Windsor bean) plants grown in water extract of bog-soil and in bog water. 1, extract; 2, bog water; 4, bog water neutralized with calcium carbonate; 5, bog water treated with carbon-black and filtered. (*After Dachnowski.*)

a soil that is unproductive for tomatoes may still produce a good crop of grain. Cultures in water extracts of unproductive soil give but poor growth, but growth is improved proportionally with the dilution of the extract with distilled water (Fig. 64). Addition of lime frequently neutralizes the toxic effect. To secure a good crop in an unproductive soil that contains toxins, it is necessary to find substances or treatments that render the soil toxins harmless.

The effects of water extract from bog-soil and those of bog water, upon the development of *Vicia faba*[1] (Windsor or broad bean), are shown in Fig. 65. The addition of calcium carbonate and the adsorptive action of carbon-black have been very effective here. In this case the toxic action of the bog water was probably due to toxins arising from the microörganisms of the soil,[2] rather than to toxins emanating from the bog plants.[k]

Toxins of some agricultural soils are organic in nature, as is indicated by the following experiment.[3] Water extract of a soil that had become alfalfa-sick was toxic to this plant, but if the soil was brought to a red heat before making the extract the latter was not toxic. Water extracts of other soils, which had not been in alfalfa culture, had no injurious effect upon the growth of this plant.

Experiments have also been made to determine the effects of various plant substances upon plant growth. Such substances are sometimes injurious and sometimes beneficial. Watering with a 3-per cent. solution of nicotin, for instance, produces good growth in tobacco, and is likewise beneficial to potatoes.[4]

[1] **Dachnowski, Alfred,** The toxic properties of bog water and bog soil. Bot. gaz. **46**: 130–143. 1908.

[2] **Löhnis, F.,** Handbuch der landwirtschaftlichen Bakteriologie. Berlin, 1910.

[3] **Pouget, I.,** and **Chouchak, D.,** Sur la fatigue des terres. Compt. rend. Paris **145**: 1200–1203. 1907.

[4] **Otto, R.,** and **Kooper, W. D.,** Untersuchungen über der Einfluss giftiger, alkaloidführender Lösungen auf Boden und Pflanzen. Landw. Jahrb. **39**: 397–407. 1910.

[k] That bog waters are toxic to ordinary plants (at least, in that they have an acid reaction), has long been suspected. Schimper (**Schimper, A. F. W.,** Plant geography upon a physiological basis. Translated by W. R. Fisher. Oxford, 1903) considers bogs as physiologically dry, but is not clear as to just what physiological dryness may be due to. Livingston tested the two logical possibilities in this case. He found (**Livingston, B. E.,** Physical properties of bog water. Bot. gaz. **37**: 383–385. 1904) that high osmotic concentration of bog water is not a possible explanation of physiological dryness; bog water has a freezing-point no lower than that of water from drained swamps and rivers of the vicinity. By the use of an alga as a physiological indicator, the same author showed very clearly that bog waters usually contain toxic substances. (**Livingston, B. E.,** Physiological properties of bog water. Bot. gaz. **39**: 348–355. 1905.) It appeared also that this toxicity (for the alga used) was surely not directly related to acidity, the degree of acidity being measured with phenolphthalein as indicator. It is interesting to note that this first step toward an analysis of the bog-water problem occurred at almost exactly the same time as the general problem of toxic substances in arable soils was opened up (in its modern sense) by Whitney and Cameron (1904) [see note 1, p. 99] and by Belford and Pickering (1903) [see note *j*, p. 99]. The three lines of work were entirely independent. Transeau also (**Transeau, E. N.,** On the development of palisade tissue and resinous deposits in leaves. Science, n. s. **19**: 866–867. 1914) had shown that bog water is toxic, to Rumex at least, before the excellent studies of Dachnowski (cited here in text), and those of Rigg were published. (**Rigg, G. B.,** The effect of some Puget Sound bog waters on the root hairs of Tradescantia. Bot. gaz. **55**: 314–326. 1913. **Idem,** The toxicity of bog water. Amer. jour. bot. **3**: 436–437. 1916. **Idem,** A summary of bog theories. Plant world **10**: 310–325. 1916.) It seems probable that microörganisms and lack of oxygen have to do with the production of these bog toxins.—*Ed.*

Summary

1. **Cultures in Artificial Media.**—The essential chemical elements for plants in general are: Carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, and iron (C, H, O, N, S, P, K, Ca, Mg, Fe). Carbon and oxygen enter ordinary green plants as carbon dioxide (CO_2), while hydrogen and oxygen enter as water (H_2O). As already seen, these two compounds are decomposed in chlorophyll-bearing cells, by the action of sunlight, forming carbohydrates ($[CH_2O]_n$) and free oxygen. From carbohydrates and other substances the plant cells form the many different organic compounds found in the plant body. Tissues without chlorophyll must absorb their carbohydrates (and often many other organic compounds from their surroundings, including the green tissues of the same plant. As has also been, seen, nitrogen enters the ordinary plant mainly as nitrates (sometimes as nitrites, ammonium salts, or organic nitrogenous substances), and these become combined with carbohydrates, etc., to form many of the most complex substances occurring in plants. When plants are completely burned all of the carbon, hydrogen, oxygen, and nitrogen are given off as gases, but there remain small amounts of many other essential and non-essential elements in the form of incombustible ash. The total ash of ordinary plants constitutes only about 5 per cent. of the total dry weight, or about 0.02 per cent. of the green weight. The other essential elements (S, P, K, Ca, Mg, Fe) of the ash are absorbed by ordinary plants from the soil, just as are water and nitrates, and the supply is in the form of inorganic salts: mainly nitrates (NO_3), sulphates (SO_4), and phosphates (PO_4), of potassium, calcium, magnesium, and iron.

The elements absorbed through the roots may be studied by artificially controlled cultures in water solutions or in pure quartz sand, etc., the latter of course containing water solution in its interstices. Numerous different solutions have been tested by many workers. A very good medium for solution cultures may be prepared with calcium nitrate [$Ca(NO_3)_2$], mono- or di-potassium phosphate, (KH_2PO_4 or K_2HPO_4), and magnesium sulphate ($MgSO_4$), about seven thousandths of a gram-molecule (the molecular weight expressed in grams) of each salt, all dissolved together in a liter of water, with addition of a very small amount (about 3 mg.) of an iron salt such as ferrous sulphate ($FeSO_4.7H_2O$). This is one of Shive's nutrient solutions. Three single-salt solutions (with trace of an iron salt) may be used in rotation, if concentrations and time periods are properly chosen.

2. **Importance of Essential Ash-constituents.**—Little is known as to just how the small amounts of essential ash constituents are used in the plant, but all must be supplied. Sulphur occurs in proteins, phosphorus in nucleins (a special group of protein-like substances), magnesium occurs in chlorophyll, and iron is essential for the formation of chlorophyll.

3. **Importance of Non-essential Ash-constituents.**—Although plants grow well with only the essential elements supplied, yet they generally contain many non-essential elements, and these are not without influence upon growth and development when they are present in the right amounts. Grasses accumulate silica in the epidermis and are thus more or less protected, from fungi, etc., by a glassy layer on the exterior.

4. **Ash Analysis of Plants.**—The chemical analysis of the ash of a plant shows what elements are present and in what proportions they occur. Different species differ in these respects, and also in the amount of total ash per unit of weight, etc. The nature of the soil influences the ash content of the plant. Different parts of the same individual plant differ in ash content. Leaves are generally richer in ash than stems and roots. The ash content alters with the age of the organ or tissue.

5. **Microchemical Ash Analysis.**—Small amounts of plant tissue may be studied by microchemical methods, to determine what chemical elements are present.

6. **The Plant and the Soil.**—Ordinary plants obtain all their ash constituents from the soil, but a chemical analysis of the soil is of little value in determining whether a plant can thrive in any given soil. The essential elements must be present as the proper salts, and these must be supplied to the obsorbing roots at proper rates. Soils may generally be much improved for growing plants by the addition of certain inorganic salts, or of material that will produce these when it is decomposed by soil microörganisms. To determine the value of a fertilizer, it must generally be actually tested with the given soil and with the kind of plant that is under consideration.

Many plant roots are normally accompanied by fungus hyphæ as *mycorhiza*, these hyphæ either forming a weft about the root or occurring in the cavities of the superficial cells. Mycorhiza is necessary for many plants, especially when growing in humus soils. It appears that the fungus hyphæ facilitate the movement of substances from the soil into the roots. There is little or no nitrification in humus soils, and it is possible that the mycorhiza in such soils may furnish the roots with some nitrogenous substances other than nitrates.

A soil may be unproductive because it contains too much (or too little) of the soluble mineral salts, or because it contains very injurious substances in toxic amounts. "Soil sickness," often resulting from repeatedly growing the same crop on the same soil, appears to furnish an example of this, the toxic materials being probably organic in such cases. They seem to be produced from the decay of plant roots, etc., or from substances emanating from the roots, and they appear often to be related to the activities of microörganisms in the soil. Such a "toxic" soil may produce good growth of one kind of plant (as wheat) while it is very injurious to another kind (as tomato). Bog soils are toxic to many forms of plants, although characteristic bog plants thrive in them.

CHAPTER V

ABSORPTION OF MATERIALS IN GENERAL

§1. **Materials Absorbed by Plants.**—We have seen in the preceding chapter that only a few inorganic materials are needed in the construction of the plant body. These essential substances are carbon dioxide, water, and certain salts containing the elements N, S, P, K, Ca, Mg, and Fe, these salts being dissolved in the soil water. From these substances [including the ten essential elements, C, H, O, N, S, P, K, Ca, Mg, and Fe] various kinds of organic compounds are built up by the green plants. Atmospheric oxygen is also absorbed by plants. Absorption of free oxygen does not generally result in an increase in dry weight, however, but is generally accompanied by the elimination of water and carbon dioxide, and thus results in a loss of plant material. Some of the organic compounds thus undergo oxidation through the respiratory process, which will be discussed later.

Some of the materials that enter the plant are commonly met with in the gaseous form (carbon dioxide and oxygen), others are generally encountered as solids (the salts of the soil, including nitrogen compounds), but they all enter plant cells as substances dissolved in water. In entering, they must all pass through the cell wall, as well as the outer layer of the protoplasm. The mechanics of the absorption of materials by plant cells is thus based upon the laws of controlling the migration of substances dissolved in other substances.[a]

§2. **Diffusion of Gases.**—If two gases are separated by a membrane permeable to them they pass through the spectum and mix. Whether there is a septum between them or not, this mixing process is termed diffusion. Two cases may be differentiated here. The first case refers to septa in which the gases are not dissolved (*e.g.*, a dry porous clay plate). The other case relates to septa in which the gases are dissolved (*e.g.*, moist animal bladder).[b] The

[a] Of course the oxygen of the air and of the soil and the carbon dioxide of the air cannot enter plant cells without being first dissolved in water; if not dissolved at a greater distance they go into solution in the water of the cell, which extends to the exterior surface of each exposed cell wall, these walls being impregnated with water of imbibition. The distinctions between solids, liquids and gases have nothing to do, primarily, with the kind of matter considered, but only with its *state*, which generally depends upon temperature. The author's presentation is here departed from to a certain extent, to avoid his apparent implication that gases enter plant cells in a manner different from that by which substances that are usually solid or liquid make their entrance.—*Ed.*

[b] The term *dialysis* refers to the process of separating two dissolved substances by letting one diffuse through a septum that is impermeable to the other—a common laboratory operation—and follows the same principles, whether the diffusing substance is commonly met with in the gas, liquid, or solid form. The word *osmosis*, frequently encountered in connection with the diffusion of substances through membranes, should be dropped, for it does not add to

velocity of diffusion of undissolved gases depends upon the density of the diffusing gas (temperature and pressure being the same) and is inversely proportional to the square root of this density. For instance, the density of hydrogen is approximately 1, while that of oxygen is 16, and the velocities of diffusion of these two gases are to each other as 1 is to 4; *i.e.*, hydrogen passes through a dry porous clay septum four times as rapidly as does oxygen when the two gases have the same temperature and pressure.

In the diffusion of *dissolved* gases the density of the gas plays no direct part. Here the velocity of the movement is directly proportional to the coefficient of solubility of the gas in the solvent contained in the septum. In the absorption of gases by plant cells, it is diffusion of dissolved gases that is encountered, since the cell walls are impregnated with water. According to the law of gas diffusion, carbon dioxide should enter plant cells more slowly than do any of the other gases encountered; on the basis of the principle of diffusion of dissolved gases, it should enter more quickly that the others, since it possesses the greatest solubility in water (and in water-impregnated membranes). Thus it happens that carbon dioxide, in spite of the small amount of it in the air, is still absorbed by plant cells in adequate amounts.[c]

§3. **Absorption of Gases.**—Plants possess various structures that favor gas absorption and gas movement, among which are stomata, lenticels, and numerous intercellular passages traversing the plant body in all directions. The

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clearness and is frequently confusing. We have two kinds of diffusion with which to deal here, one being the intermingling of gases as such and the other that of substances (such as carbon dioxide, alcohol, potassium nitrate, etc.) while dispersed (dissolved) in a solvent; the solvent is usually liquid (water), but substances may dissolve in solid material—as carbon dioxide in the wax-like, cuticular material of many exterior cell walls. Diffusion of undissolved gases is met with in the inward and outward movement of water vapor, carbon dioxide and oxygen through stomatal openings and from place to place in the plant body through *gas-filled* intercellular spaces, but gases do not pass through the cell walls or protoplasm of active cells, and therefore cannot get inside the cells, unless they are first dissolved, usually in water. (See below, in text.) Of course, when water vapor is dissolved in liquid water it simply becomes a part of the liquid, being *condensed* from the gaseous to the liquid state. This and the following paragraphs have been subjected to some modification, in accordance with these principles. It may be added at this point that, besides the diffusion of gases and that of dissolved substances, there is another kind of movement met with in plants, namely that of molar streaming. This occurs with gases and liquids and also (but not commonly in the plant) with suitably sub-divided solids (as sand). When a gas or liquid is forced through openings, by pressure, it is this molar movement that has to be considered. Diffusion may go on at the same time, in the liquid or gas stream, its direction being independent of the direction of the streaming. If diffusion and streaming are in the same direction, the *rate of movement* is the sum of the rates of diffusion and streaming; if they are in opposite directions the difference is the rate of movement.—*Ed.*

[c] Carbon dioxide is about three times as soluble in water as is oxygen. It is as a gas, however (undissolved in either liquid or solid), that carbon dioxide first enters the ordinary green plant through stomatal openings. See: **Blackman,** 1895. [See note 2, p. 36.] **Brown,** 1899. [See note 1, p. 34.] **Brown** and **Escombe,** 1900. [See note 1, p. 34.] Having entered by gas diffusion, carbon dioxide soon passes into solution in the water with which the cell walls abutting on the sub-stomatal intercellular spaces are impregnated, and it diffuses as a dissolved substance through these walls and into the cells.—*Ed.*

migration of gases through different kinds of plant septa has been investigated by many authors. The most recent and extensive studies on the molar or streaming movement and the diffusion of gases through plant cell walls are due to Wiesner and Molisch.[1] In these experiments a piece of dry plant tissue was fastened over one end of a straight glass tube (6 mm. in internal diameter and 50 to 100 cm. long) with sealing wax, and the joint was then covered with a mixture of equal parts of resin and beeswax. When soft, succulent tissues were employed, the tissue was kept in place by a perforated metal cap, and was kept from being crushed by rubber rings, the openings of which just fitted the end of the glass tube. The tube was partly or entirely filled with mercury and the open end was closed with the finger while the tube was inverted, the open end being then placed in a vessel of mercury. The tube was finally arranged in an upright position, with the mercury below. After a number of days the height of the mercury column in the tube was measured.

An experiment with birch bark may serve as an example. A piece of white periderm, 0.09 mm. thick, was used. The height of the mercury column in the tube was 400 mm. at the beginning of the experiment and remained the same, after fourteen days, the usual corrections for temperature and pressure having been applied. Wiesner and Molisch came to the following conclusions from the result of these experiments.

1. Plant cell walls, either wet or dry, whether the cells are alive or dead, are impermeable to the molar movement of gases under ordinary pressures.

2. Protoplasm and cell sap are likewise impermeable to this kind of gas movement, so that there is no movement of air as such through tissue without intercellular passages. This experiment explains how negative gas pressure (*i.e.*, pressure less than that of the surrounding atmosphere) in wood may be maintained, which will be discussed later.

Similar tubes filled partly with mercury and partly with various gases were employed in experiments upon the *diffusion* of gases through dry and moist plant membranes. The velocity of outward diffusion was indicated by the rate of rise of the mercury column in the tube. An experiment with periderm of the potato tuber may be taken as an example. Two tubes were filled with carbon dioxide, one being closed with a dry, the other with a moist piece of periderm. In the tube with the dry membrane the mercury rose only 5 mm. during a period of thirty days, while the tube with moist membrane showed a corresponding rise of about 40 mm. This experiment shows that the interchange of gases through the wet membrane occurred according to the principles of diffusion of dissolved gases; carbon dioxide passed outward through the membrane more rapidly than air passed inward, thus causing the mercury to rise in the tube. If the septa to be studied were permeable to, but did not dissolve the gas (as in the case of a dry porous clay plate), then, according to the law of gas diffusion, the mercury column should fall. From a series of experiments similar to this, these authors came to the following conclusions:

[1] Wiesner, J., and Molisch, H., Untersuchungen über die Gasbewegung in der Pflanze. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien. 98*I*: 670–713. 1890.

1. Gases move through cell walls only in solution in the water imbibed in the wall; when intercellular spaces are present, they of course facilitate the movement through the tissue.

2. Gases pass through cell walls the more easily, the more thoroughly the latter are impregnated with water. Diffusion is most rapid through cell walls of algæ and, in general, through those of submerged plant parts.

3. Cell walls that are neither lignified nor suberized do not permit the passage of some gases when the walls are dry, but carbon dioxide and oxygen pass through practically dry walls if the latter are lignified or ssuberized.[d]

These experiments suggest an important ecological consideration as regards suberization and cutinization in plant tissues. If the entire surface of the plant were covered by a dry membrane of pure cellulose, then the interior cells would be suffocated, but the presence of cork and cutin, in the absence of lenticels and while the stomata are closed, protects plants from desiccation without at the same time preventing gaseous exchange.

4. Carbon dioxide passes out of plant cells more rapidly into air than into water.

Since Wiesner's experiments indicate that gases may pass through the cuticle, the question arises, to what extent do open stomata increase the rate of gaseous exchange through the epidermis? To answer this question F. F. Blackman[1] constructed a special apparatus described below (Fig. 66). Two brass rings, each prolonged into two tubes at opposite points and each with a glass plate attached to one side, were used as gas chambers, each chamber being about 5 mm. deep and 36 mm. broad. A leaf was clamped between two chambers of this kind and the joints were sealed with wax. Oblong chambers were used for narrow leaves (Fig. 66, *A*). Gas of known composition was passed simultaneously, but separately, through both chambers and then analyzed. Experiments with leaves having stomata only on the lower surface showed that the respiratory gas exchange occurred almost entirely through these openings. For example, a leaf of *Nerium oleander* gave out 0.002 g. of CO_2 from its upper surface while 0.065 g. escaped from the lower; thus the two sides gave off this gas in the ratio of 3 to 100.

Further experiments upon the assimilation of carbon dioxide in light showed that leaves absorb this gas from the air almost exclusively through the stomata. Leaf surfaces without stomata practically fail to absorb carbon dioxide. When the lower surface alone is provided with stomata, coating this surface with petrolatum greatly decreases gaseous exchange without wholly stopping it, as Mangin has shown (see page 35). When stomata occur on both sides of the

[1] **Blackman, F. F.**, 1895, No. II. [See note 2, p. 36.]

[d] Molar movement of gases can occur only through intercellular spaces and relatively large openings in plant membranes (stomatal openings, etc.), and gas diffusion can occur through such openings and through dry membranes with relatively large pores (porous porcelain, etc.). The diffusion of dissolved gases is possible if the gas is soluble in the membrane. When the latter contains water this kind of diffusion can occur, for the gas dissolves in the water. When the membrane contains little or no water, but contains suberin, etc., the action is similar to that of a wet membrane, if the gas dissolves in the wax-like material as it does in water.—*Ed.*

leaf, the amount of carbon dioxide absorbed is greater on the side where these openings are most abundant. In the case of *Alisma plantago*, the number of stomata on the upper is to the number on the lower surface as 135 is to 100. While the upper surface was absorbing 0.10 or 0.15 g. of the gas the lower surface absorbed 0.06 or 0.11 g.

These experiments led Brown and Escombe[1] to carry out the following interesting investigations. The Catalpa leaf has stomata only on the lower surface, through which carbon dioxide is absorbed in the presence of light. Under the most favorable conditions 700 cc. of this gas is absorbed per hour, per square meter of leaf surface. If it is assumed that absorption proceeds equally over the entire leaf surface, then each molecule of carbon dioxide enters the leaf with an average velocity of 3.8 cm. per minute. This velocity is only half of that with which carbon dioxide is absorbed by the exposed surface of a sodium

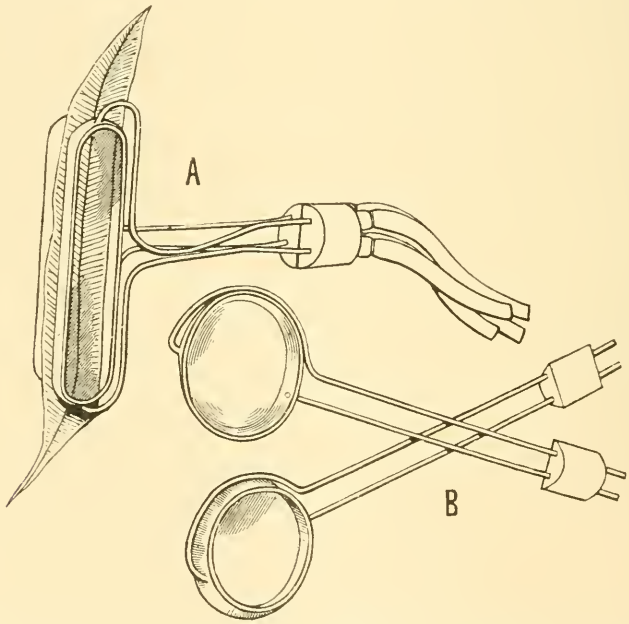

FIG. 66.—Apparatus for the study of gaseous exchange through the upper and lower surfaces of leaves. (*After Blackman.*)

hydroxide solution. But since the gas is absorbed only through the stomata, and since the total area of the stomatal openings is not greater than one-one-hundredth of the entire leaf surface, then a surprisingly large number (380 cm.) is obtained as the average velocity of absorption of carbon dioxide through the stomata. This number is fifty times as great as that representing the absorption of CO_2 by the free surface of sodium hydroxide solution. These results led to the following experiment. Test-tubes were filled with aqueous solution of sodium hydroxide and covered with thin, perforated plates, different plates

[1] **Brown**, 1899. [**Brown** and **Escombe**, 1900.] [See note 1, p. 34.]

having openings of different diameters. Some of the results are tabulated below. The velocity of carbon dioxide diffusion was found to be proportional, not to the area of the opening in the plate, but to its diameter.

Diameter of Opening	Diffusion of CO_2		Ratio of Areas of Openings	Ratio of Diameters of Openings	Ratio of Amounts of CO_2
	Per Hour	Per Hour, per Square Centimeter			
mm.	*cc.*	*cc.*			
22.70	0.2380	0.0588	1.000	1.000	1.00
6.03	0.0625	0.2186	0.070	0.260	0.26
3.23	0.0399	0.4855	0.023	0.140	0.16
2.12	0.0261	0.8253	0.008	0.093	0.10

While the area of the smallest opening (diameter 2.12 mm.) was less than a hundredth of that of the largest (diameter 22.7 mm.), the amount of gas passing the former was one-tenth, rather than one-hundredth, of the amount passing the latter. From this it follows that if a vessel of sodium hydroxide solution is covered with a thin plate perforated with very small openings, the quantity of carbon dioxide absorbed may be as great as though no cover were present at all. The total area of all the openings may be only a small fraction of the total surface of the plate, however. It was found that diffusion was most rapid when the distances between the openings were each ten times as great as the diameter. This proportion holds approximately for the distribution of stomata in most leaves. Therefore the velocity of gas absorption is as great when the stomata are open as it would be if no cuticle were present and if the whole leaf were covered with a wet membrane of pure cellulose.

Investigations of movements of gases in water plants[1] have shown that the air of the intercellular spaces has about the same composition as that of the external atmosphere.

§4. Diffusion of Dissolved Substances.[2]—Many substances that are not gases at ordinary temperatures are soluble in water, but not all substances are appreciably so; oils, for example, are generally practically insoluble in water.[e]

[1] **Devaux, Henri,** Du mécanisme des échanges gazeux chez les plantes aquatiques submergées. Ann. sci. nat. Bot. *VII* 9: 35–179. 1889.

[2] **Dastre, M. A.,** Traité de physique biologique 1: 466. Paris, 1901.

[e] The following discussion of osmotic pressure and related phenomena is largely due to the editor, but the spirit and apparent intent of the author is followed as closely as possible, at the same time avoiding the author's curious conceptions that dissolved substances are liquids and that "osmosis" and diffusion are essentially different. For another attempt at presenting these phenomena to the student of physiology, see: **Livingston, B. E.,** The rôle of diffusion and osmotic pressure in plants. Chicago, 1903. Also see: **Findlay, Alexander,** Osmotic pressure. London, 1913. **Washburn, Edward W.,** An introduction to the principles of physical chemistry. 2d ed. 516 p. New York, 1921. The last-named discussion is the most thorough from the physical and mathematical point of view.—*Ed.*

Whether the dissolved substance is a gas, liquid or solid under ordinary conditions, it forms an aqueous *solution* when it is dissolved in water. The dissolved substance is usually called the *solute* and the water in which it dissolves is the *solvent*. A solution may contain many different kinds of solutes, all dissolved in the common solvent. All dissolved substances diffuse in all directions within the limits of the solution or solvent, and tend to become equally distributed throughout its volume. If two solutions having a common solvent but different solutes be brought into contact, the two solutes diffuse into each other's region and they eventually become completely mixed, so as to form a single solution of two solutes. The solvent itself exhibits a corresponding tendency to diffuse in all directions; if a mass of pure water be brought into contact with an aqueous solution, water enters the solution and dilutes it, while the solute or solutes enter the water and convert it into a solution, this process continuing until the resulting solution becomes uniform throughout. (If the solute be another liquid—as alcohol, glycerine, etc.—the solute may become the solvent when it predominates. Thus we may have a solution of glycerine in water or a solution of water in glycerine, etc.). It appears that the solute and solvent attract each other and that the latter enters between the particles of the former, thus hastening their outward diffusion. If a membrane that is permeable to water but relatively impermeable to the solute be placed around the solution and be, in turn, surrounded by the pure solvent, a pressure, called *osmotic pressure*, is developed, which tends to drive the membrane outward before the outwardly diffusing solute, thus stretching—or even rupturing—the membrane. This phenomenon of osmotic pressure was discovered by Dutrochet,[1] as early as 1827, who observed the escape of zoospores from an alga and tried to arrive at an explanation for the bursting of the sporangium. He supposed that an increased absorption of water by the sporangium was brought about by water-attracting substances within, and that this caused the rupture. If an animal bladder filled with aqueous sugar or salt solution is placed in water, the solvent enters, and the outwardly directed osmotic pressure simultaneously developed may become so great as to rupture the membrane itself. The rupture of the alga sporangium as observed by Dutrochet, was caused in a similar way [f]

[[1] **Dutrochet, René Joachim Henri,** Nouvelles observation sur l'endosmose et l'exosmose, et sur la cause de ce double phénomène. Ann. chim. et phys. 35: 393–400. 1827.]

[f] It is still commonly stated or implied that the entering water turns on itself after entrance, and, thus tending to return, presses outwardly upon the membrane and causes the rupture. But the bladder membrane is, in itself, as permeable to water diffusing in one direction as to the same substance diffusing in the other, and more water enters than passes out, so that if there is a pressure of water in either direction it should tend to collapse the bladder, not to explode it from within. A logical picture may represent the osmotic pressure causing the rupture as directly due to a tendency of the solute particles (as of sugar or salt, or ions), or of any combinations of solute particles with water particles (in so far as these are unable to pass the septum), to diffuse outward into the surrounding solvent. This, in turn, may be considered as brought about or made possible by the entrance of water (at least it cannot occur without this entrance), which, finally, may be due to an attraction exerted upon the water by the solute. Such a simple picture may still serve the purposes of physiology, although serious complications appear to arise sometimes when a complete appreciation of osmotic and related phenomena is attempted. The most thorough discussion of osmotic pressure so far available is that given by Washburn [see note *c*, p. 109].—*Ed.*

Brücke (1843) advanced a theory of diffusion through septa, based upon the observation that if two liquids are separated by a membrane, the one that wets the membrane more thoroughly (*i.e.*, in which the latter swells more rapidly) penetrates more rapidly. For example, if a membrane of rubber or collodion be employed, then alcohol passes through more rapidly than water, but with a membrane of animal bladder the opposite is true. Rubber and collodion membranes imbibe alcohol more rapidly than water and they also swell more in alcohol. Thus alcohol passes through such septa more rapidly. But animal bladder swells in water and shrinks in alcohol, and water passes through such a membrane more quickly than does alcohol. Animal bladder swells more in pure water than in salt solution, and the former passes through such a septum more rapidly than does the latter. These facts indicate that the water is more forcibly attracted by the membrane substance than are the salts, so that the concentration of the imbibed solution in the pores of the membrane increases as the distance from the pore walls becomes greater. Ludwig[1] has shown further that if dry pieces of animal bladder are placed in a solution of sodium sulphate or sodium chloride, the solution that is imbibed is less concentrated than what remains. By means of a hand press he pressed some of the liquid out of such impregnated pieces of bladder, and found that the expressed solution possessed a concentration higher than the average concentration of the solution originally within the pores of the bladder.[g]

Osmotic pressure is studied by various kinds of osmometers. Baranetskii's[2] osmometer consists of two chambers separated by a membrane, one containing a salt solution, which is to increase in volume, while the other contains water introduced through a funnel that is attached by a rubber tube. As the solution increases in volume a rubber-tube outlet from the solution chamber allows the overflow to be caught in a graduated flask. The surface of the water in the funnel must be kept at the same height as that of the solution in the exit tube. The movement of liquids through the membrane continues until the concentration of the two solutions is the same on both sides.

Experiments upon diffusion of dissolved substances through membranes have shown that all water-soluble substances may be classified into two groups according to their relation to the membrane, those which can pass through the membrane (crystalloids) and those which cannot (colloids). Upon these different properties of colloids and crystalloids depends the method of dialysis, by which colloid material may be separated from crystalloids. Many plant substances are colloids and they cannot, therefore, diffuse out of the cells.[h]

[1] **Ludwig, C.,** Ueber die endosmotischen Aequivalente und die endosmotische Theorie. Poggendorff's Ann. Phys. u. Chem. 154 ("der ganzen Folge"): 307–326. 1849.

[2] **Baranetskii, I.** Investigations on diosmosis as related to plants. [Russian.] Inaug. Dissertation. St. Petersburg, 1870. **Barenetzky, J.,** Diosmotische Untersuchungen, Poggendorff's Ann. Phys. u. Chem. 223 ("der ganzen Folge"): 195–245. 1872.

[g] These considerations give the reason why the membrane is more permeable to one substance than to the other, or, they merely state this fact in other terms.—*Ed.*

[h] But the matter is not so simple as this. Many water-soluble crystalloids fail to pass certain membranes that are permeable to water, and some colloids do pass them. Colloids and crystalloids are difficult to distinguish accurately, these terms referring to the state rather than

Membranes of animal bladder, parchment paper and collodion, as well as the so-called precipitation-membranes, are all used for osmotic experiments. Cellulose membranes, giving the cellulose reaction with zinc chloride and iodine (Baranetskii, 1870) can be produced by treatment of collodion membranes with ferric chloride. Of the above-mentioned membranes, animal bladder is much like the plant cell wall in its osmotic properties, while precipitation membranes are only very slightly permeable to many substances and can give rise to high osmotic pressures. Suitable supports must be provided for these delicate membranes. Pfeffer[1] employed porous clay cylinders such as are used in electric batteries. When such a porous cell is filled with a copper sulphate ($CuSO_4$) solution and placed in a solution of potassium ferrocyanide ($K_4Fe(CN)_6$), a membrane of copper ferrocyanide ($Cu_2Fe(CN)_6$) is precipitated in the porous wall. Similar precipitation membranes may be obtained with other substances, such as iron silicate. To measure osmotic pressure the porous cylinder, with its membrane, is filled with the solution to be studied and is connected with a mercury manometer, the cylinder being submerged in water (Fig. 67). The magnitude of the pressure exerted at equilibrium is then read upon the manometer.[i]

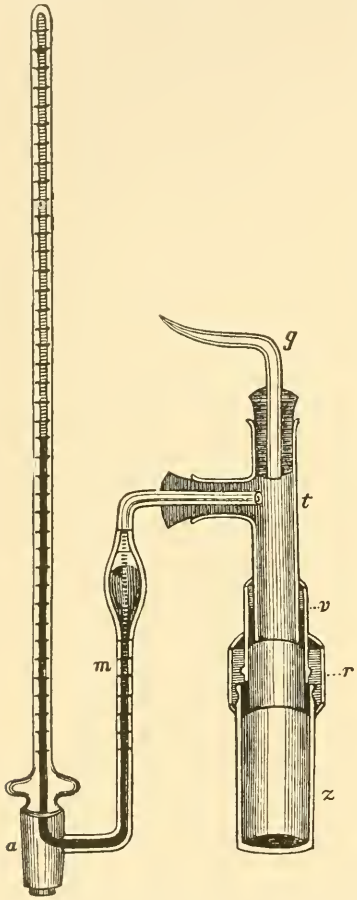

FIG. 67.—Pfeffer osmometer (z), with closed mercury manometer. (*After Pfeffer.*)

to the nature of the substance considered. In this connection see: **Weimarn, P. P. von,** Grundzüge der Dispersoidcheme. 127 p. Dresden,. 1915. For a clear and very readable discussion of colloids in general, see· **Ostwald, Wolfgang,** Die Welt der vernachlässigten Dimensionen. x+219 p. Dresden and Leipzig 1915. Also see: **Hatschek, Emil.** An introduction to the physics and chemistry of colloids. 4th ed. 172 p. London, 1922. Other books on this subject are mentioned in the List of Books, p. xix.—*Ed*

[1] **Pfeffer, W.,** Osmotische Untersuchungen. Leipzig 1877.

[i] The most perfect precipitation membranes yet made are those of Morse and his coworkers, who have been engaged for many years in very thorough studies on the osmotic pressures developed by concentrated solutions. This work has been carried out in the Chemical Laboratory of the Johns Hopkins University. Much improved forms of the Pfeffer cell have been employed and the copper ferrocyanide membranes of these writers have proved quite impermeable to cane sugar for many days, even with very high pressures. For accounts of this work see: **Morse, H. N.,** and **Horn, D. W.,** The preparation of osmotic membranes by electrolysis. Amer. chem. jour. **26**: 80–86. 1901.

Walden[1] obtained semi-permeable precipitation membranes in the following manner. The upper end of a glass tube 5 cm. long and 1 cm. wide is closed by the finger and the lower end is dipped into a solution containing 50 g. of water, 10 g. of gelatine, and 1 g. of ammonium chromate. When the tube is lifted from the solution, the lower end remains closed by a thin membrane, which is rendered insoluble in water by the action of light. A precipitation membrane of copper ferrocyanide is then deposited in the hardened gelatine film, according to the method employed by Pfeffer.

Experiments with precipitation membranes have given the general results summarized below. Other conditions remaining the same:—

1. Osmotic pressure is proportional to the concentration of the solution. Thus 1-, 2- and 4-per cent. solutions of cane sugar developed osmotic pressures equivalent to 53.2 cm., 101.6 cm. and 208.2 cm. of a mercury column, respectively.

2. Osmotic pressure increases with rise in temperature. A 1-per cent. saccharose solution at temperatures 6.8°, 13.7° and 22°C. gave osmotic pressures of 50.5 cm., 52.5 cm. and 56.7 cm. of a mercury column, respectively.

3. Osmotic pressure depends upon the nature of the dissolved substance. Six-per cent. solutions of (1) gum arabic, (2) gelatine, (3) saccharose and (4) potassium nitrate gave osmotic pressures of (1) 25.9 cm., (2) 23.8 cm., (3) 287.7 cm. and (4) 700 cm. of a mercury column, respectively. Colloids (such as gum arabic and gelatine) thus produce much lower osmotic pressures than do crystalloids.[i]

4. Osmotic pressure depends upon the nature of the membrane. Six-per cent. solutions of the four substances named above gave the following osmotic pressures (in centimeters of a mercury column) with membranes of copper ferrocyanide, parchment paper and animal bladder, respectively.

Morse, H. N., The osmotic pressure of cane sugar solutions at high temperatures. *Ibid.* **48**: 29–94. 1912. **Idem,** The osmotic pressure of aqueous solutions. Carnegie Inst. Wash. Pub. 198. 222 p. 1914. During the same period other very important experimental studies on the osmotic pressure developed by concentrated solutions have been prosecuted by Berkeley and Hartley, in England. See: **Berkeley, Earl of,** and **Hartley, E. G. J.,** On the osmotic pressure of some concentrated solutions. Phil. trans. Roy Soc. London **A206**: 481–507. 1906. For a general discussion, see **Findlay,** 1913, also **Washburn,** 1921. (See note *e*, p. 109.)—*Ed.*

[1] **Walden, Paul,** Ueber Diffusionserscheinungen an Niederschlagsmembranen. Zeitsch. physik. Chem. **10**: 699–732. 1892.

[i] As is brought out a little farther on, the concentration of the solutions should not be stated in terms of percentage for such comparisons; they should be given in terms of a volume-molecular, or still better, of a weight-molecular solution. The former gives the number of gram-molecules of solute dissolved in a liter of solution (at a stated temperature) and the latter gives the number of gram-molecules of solute dissolved in 1000 g. ($\frac{1000}{18}$ = 55.56 g.-mol.) of water taken as H_2O. For a valuable discussion of the relation of volume-molecular and weight-molecular solutions to physiological considerations, see: **Renner, O.,** Ueber die Berechnung des osmotischen Druckes. Biol. Centralbl. **32**: 486–504. 1912. The general principle holds, as stated in the text, however. See also note *n*, below (p. 123).—*Ed.*

Substance, in 6-Per Cent. Solution	Kind of Membrane		
	Copper Ferrocyanide	Parchment Paper	Animal Bladder
	cm. Hg	*cm. Hg*	*cm. Hg*
Gum arabic	25.9	17.7	14.2
Gelatine	23.8	21.3	15.4
Saccharose	287.7	29.0	14.5
Potassium nitrate	700.0	20.4	8.9

The crystalloids, saccharose and potassium nitrate, produced lower pressures than did the colloids, gum arabic and gelatine, when plant or animal membranes were used. This seems to be in disagreement with statement 3, above, but it is explained by the fact that these two crystalloids readily pass through such membranes, while the precipitation membranes are almost impermeable to them.

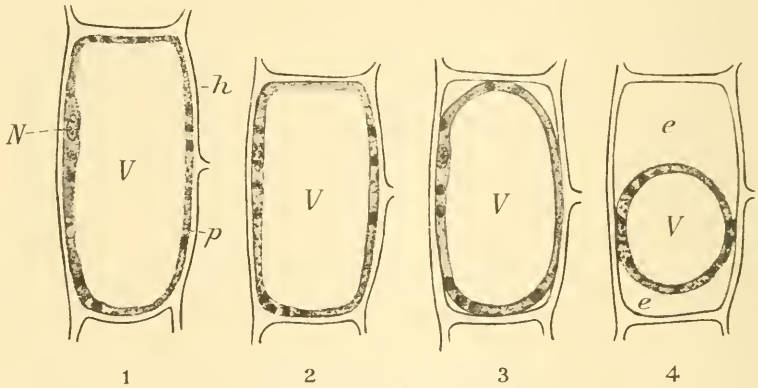

Fig. 68.—Successive stages of plasmolysis. *N*, nucleus; *V*, vacuole. (*After deVries.*)

Pfeffer's experiments indicated that, other conditions remaining the same, the magnitude of the osmotic pressure differed according to the nature of the dissolved substance, and the question arose whether this phenomenon obeyed any law. This question was answered by deVries,[1] who used living plant cells instead of the artificial cells employed by Pfeffer. He determined the isosmotic (or isotonic) coefficients of various substances by means of the *plasmolytic* method.

As is well known, plasmolysis occurs when a living plant cell is placed in a sufficiently strong (10-per cent.) solution of such substances as cane sugar, sodium chloride, etc. At first there is a decrease in cell volume, to a certain point, after which the protoplasm separates from the cell wall and withdraws inward (Fig. 68). The cell gradually regains its earlier form if the salt solution is

[1] **Vries, Hugo de,** Eine Methode zur Analyze der Turgorkraft. Jahrb. wiss. Bot. 14: 427–601. 1884.

replaced by water. Cells with colored sap are very good for plasmolytic experiments, since the coloring matter is retained within the shrinking vacuole, leaving the space between the protoplasm and the cell wall filled with colorless solution. By the use of such cells plasmolysis may be readily detected, even in its incipient stages. DeVries used mature cells with colored sap and determined the concentration of the plasmolyzing solution when the latter was just strong enough to cause separation of the protoplasm from the wall at the corners of the cell (Fig. 68, 3). If no further contraction of the protoplasm occurs it follows that the osmotic pressure within the vacuole just equals that of the external solution. The same experiment was repeated with various substances, and the limiting concentration (*i.e.*, that concentration which is just strong enough to cause incipient plasmolysis) was determined for each. In this way concentrations of various substances were found that produced the same osmotic pressure with the same membrane. Such solutions are termed *isosmotic* or *isotonic*.

The colored epidermal cells of the leaf sheath of *Curcuma rubricaulis*, of the leaves of *Tradescantia discolor*, and of the petiolar scales of *Begonia manicata*, are all very well suited to such experiments as that just described. Twelve preparations may be made for each experiment, six being placed in various concentrations of the substance to be studied, and the other six in corresponding concentrations of potassium nitrate. All preparations must be taken from the same region of the leaf or other plant organ. To accomplish this, a narrow rectangle is marked on the leaf, and divided longitudinally into halves and transversely into six divisions, the area of each of the resulting sections being about 1 sq. mm. Each piece of epidermis is removed with a razor and placed in a glass cylinder (about 10 cm. tall and 2 cm. in diameter[k]) containing the solution to be tested. The cylinders are loosely stoppered to prevent evaporation, and the preparations are left in the solutions about two hours.

Volume-molecular solutions were employed, containing the molecular weight of the solute in grams (called a gram-molecule or a mol)[1] per liter of solution. [See note *j*. p. 113.] A volume-molecular solution (*m*) of potassium nitrate contains, for example, 1 g.-mol. (101.1 g.) of the salt in a liter of solution, and a tenth-molecular solution (0.1 *m*.) contains 10.11 g. of the salt per liter. In physiological studies it is generally more convenient to calculate solution concentrations as gram-molecules per liter than to consider them in terms of percentage.

DeVries compared the osmotic pressures developed by equimolecular solutions of various substances, and found that the substances tested fell into four groups according to the amount of pressure developed, the four different pressures obtained being, relatively, 0.066, 0.100, 0.133, and 0.166. The second group represents the pressure caused by potassium nitrate. These numbers are approximately in the proportion of 2 : 3 : 4 : 5, so that if the pressure produced

[1] **Ostwald, Wilhelm,** Lehrbuch der allgemeinen Chemie. 2te Aufl. 2: 212. Leipzig, 1906. [Idem, Outlines of general chemistry. Translated by James Walker. London, 1895.]

[k] Much shorter vials are more convenient, about 1 cm. in diameter and 2 cm. high.—*Ed.*

by a volume-molecular solution of potassium nitrate be considered as 3, then the pressure developed by a volume-molecular solution of any other substance not in the same group is 2, 4, or 5, according to the group in which the given substance belongs. On this account deVries adopted as his unit of osmotic pressure one-third of the pressure produced by a volume-molecular solution of potassium nitrate, so that a volume-molecular solution of this salt, or of any other salt belonging to the same group, always produced a pressure of 3, and the three other groups of substances gave pressure of 2, 4 and 5, respectively. The numbers 2, 3, 4 and 5 were termed isosmotic coefficients; they represent the relative osmotic pressures developed by equimolecular solutions of the various substances.

The isosmotic coefficients were determined in the following manner. Three cane sugar solutions, 0.20-, 0.22- and 0.24-volume-molecular, and three solutions of potassium nitrate, 0.12-, 0.13- and 0.14-volume-molecular, were employed, for plasmolytic experiments with epidermal cells of *Curcuma rubricaulis*. Each experiment lasted seven hours. The results obtained in three such tests are given in the following table, where *n* denotes that no plasmolysis occurred, *hp* denotes that about half of the cells were plasmolyzed and *p* denotes that most of the cells were plasmolyzed. *IC* denotes the isosmotic concentration, taken to be osmotically equal to the cell sap. Volume-molecular concentration is denoted by *m*.

Experiment no.	Saccharose				Potassium Nitrate				Ratio of IC_2 to IC_1
	0.20*m*	0.22*m*	0.24*m*	IC_1	0.12*m*	0.13*m*	0.14*m*	IC_2	
				m				*m*	
1	*n*	*hp*	*p*	0.22	*n*	*hp*	*p*	0.130	0.591
2	*n*	*p*	*p*	0.21	*n*	*p*	*p*	0.125	0.595
3	*n*	*p*	*p*	0.21	*n*	*p*	*p*	0.130	0.619
				Average ratio					0.602

Since the osmotic pressure produced by a volume-molecular potassium nitrate solution is taken as 3, the numbers in the last column are to be multiplied by 3, and the average ratio thus becomes 1.81, which is the isosmotic coefficient of saccharose when that of potassium nitrate is considered as 3.

A list of substances thus tested by deVries is given in the next table, together with their isosmotic coefficients, as actually derived from experiment and also in round numbers. The next to the last column gives the percentage concentrations thus found to be isosmotic with a one-tenth volume-molecular solution of KNO_3, and the last column gives the osmotic pressure produced by a 1-per cent. solution of each substance.

Substance	Chemical Formula	Molecular Weight	Isosmotic Coefficient		Concentration Isosmotic with 0.1 *m* KNO_3	Osmotic Pressure Produced by 1.0-Per Cent. Solution
			Observed	In Round Numbers		
					per cent.	*atmospheres*
Saccharose	$C_{12}H_{22}O_{11}$	342.0	1.88	2	5.13	0.69
Glucose	$C_6H_{12}O_6$	180.0	1.88	2	2.70	1.25
Glycerine	$C_3H_8O_3$	92.0	1.78	2	1.39	2.54
Citric acid	$C_6H_8O_7$	192.0	2.02	2	2.88	1.23
Oxalic acid	$C_2H_2O_4$	90.0	....	2	1.35	2.62
Potassium nitrate	KNO_3	101.0	3.00	3	1.01	3.50
Ammonium chloride	NH_4Cl	53.5	3.00	3	0.53	6.67
Potassium sulphate	K_2SO_4	174.0	3.90	4	1.30	2.72
Magnesium sulphate	$MgSO_4$	120.0	1.96	2	1.80	1.93
Magnesium chloride	$MgCl_2$	95.0	4.33	4	0.71	4.98
Potassium citrate	$K_3C_6H_5O_7$	306.0	5.01	5	1.84	1.92

In the above table the isosmotic coefficients are seen to be about 2, 3, 4 and 5. If the coefficient for saccharose and the other organic compounds be taken as unity, then the remaining ones become $3/2$, 2, and $5/2$.

It is also evident from this table that the osmotic pressures produced by the non-electrolytes (saccharose, glycerine and the other organic compounds) are related to their molecular weights. A solution containing 92 g. of glycerine per liter produces the same osmotic pressure as one of cane sugar containing 342 g. per liter. These two solutions contain very different amounts of substance by weight, but they contain equal numbers of molecules (*i.e.*, they are equimolecular). Here all molecules produce the same osmotic pressure, and the osmotic pressure of a solution is thus proportional to its molecular concentration. This agrees with Avogadro's law for gases, which states that gas pressure is proportional to the number of molecules occurring in a given volume. Van't Hoff compared solutions of solid bodies in liquids, with gases, and concluded that osmotic pressure follows the same law as does gas pressure. One gram-molecule of any gas (*e.g.*, 44 g. of CO_2) occupies a volume of 22.4 l., with a pressure of 760 mm. and at a temperature of 0°C. When this volume of gas is reduced to 1 l., the pressure becomes 22.4 atmospheres. If the van't Hoff theory is correct, a molecular solution of cane sugar containing 342 g. per liter, should produce 22.4 atmospheres of osmotic pressure, and a 1-per cent. solution of the same substance should give an osmotic pressure of 0.69 atmospheres at 15°C. The pressure actually produced by a 1-per cent. solution of cane sugar lies between 0.62 and 0.71 atmospheres according to Pfeffer's measurements, which constitutes a brilliant confirmation of the theory.

The following table gives a summary of other osmotic values for cane-sugar solutions, as observed by Pfeffer and as calculated by the van't Hoff theory.

Concentration of Cane Sugar	Osmotic Value	
	Observed	Calculated
per cent.	*atmospheres*	*atmospheres*
1.0	0.664	0.665
2.0	1.336	1.336
2.5	1.997	1.639
4.0	2.739	2.742
6.0	4.046	4.050

It is different with electrolytes; from the table given on page 117 it is clear that, of the crystalloids, isosmotic solutions of electrolytes (metallic salts) and non-electrolytes are not equimolecular, the molecular concentrations of the former being much lower. Furthermore, there is no constant relation between the isosmotic concentrations of solutions of electrolytes on the one hand and of non-electrolytes on the other, so that electrolytes do not agree with the gas-pressure theory of osmotic pressure. For example, a 0.1-volume-molecular solution of KNO_3 ought, according to this theory, to give a pressure of 0.235 atmospheres, but it actually gives one of 0.352 atmospheres. If the value derived directly from the van't Hoff theory be multiplied by $3/2$, the isosmotic coefficient of this salt (considering the coefficient of cane sugar as unity), the value 0.352 is obtained, which is the same as that found experimentally. Equimolecular solutions of potassium nitrate and of organic substances are thus not isosmotic. To obtain a solution of potassium nitrate that shall produce the same osmotic pressure as does a 0.1-molecular cane-sugar solution it is necessary to prepare a $1/15$ ($2/3 \times 1/10$) molecular solution of the salt. Salts with other isosmotic coefficients must be employed in corresponding concentrations. Thus, a 0.05-molecular solution of potassium sulphate is isosmotic with a 0.1-molecular solution of cane sugar. The osmotic pressure of a weak solution of an electrolyte is thus equal to the theoretical pressure multiplied by the isosmotic coefficient of the electrolyte in question. This departure from the theory is explained by Arrhenius' hypothesis, which supposes that electrolytes in solution *dissociate* into *ions*. In a sodium chloride solution, for example, sodium and chlorine ions are both present as well as molecules of sodium chloride. The more dilute the solution, the greater is the degree of dissociation.

According to the Arrhenius theory of electrolytic dissociation, the isosmotic coefficient of potassium nitrate indicates that the number of *particles* in a solution of this salt is increased by dissociation, and if half of the molecules be considered as dissociated the total number of particles ought to be $3/2$ of what it would be without dissociation, and the osmotic pressure should be correspondingly increased. A dissociated molecule of KNO_3, in the form of two ions, K and NO_3, produces twice as much osmotic pressure as does an undissociated molecule.

Potassium sulphate has an isosmotic coefficient of 2 at the concentrations employed by de Vries, the molecule of this electrolyte dissociates into three ions, K, K and SO_4, and the coefficient 2 indicates, in this case also, that half the total number of molecules are to be considered as dissociated. The number of particles in solution would thus be about doubled, for $\frac{1}{2} + 3 \times \frac{1}{2} = 2$.[l]

DeVries used salt solutions of about 0.1-volume-molecular concentration, these being about half dissociated. The degree of dissociation varies with the concentration, and so the osmotic coefficients obtained by deVries cannot be used for solutions of other concentrations, the coefficients for which must be obtained through the use of isosmotic solutions,[1] employing a solution of an undissociated and unhydrated substance as a standard.

Errera[2] proposed the *myriotonie* as a unit for the measurement of osmotic pressure, to replace the arbitrary one of an atmosphere. A *tonie* is the pressure exerted upon a surface of 1 sq. cm. by 1 dyne (the well-known unit representing the force necessary to give a velocity-acceleration of 1 cm. per second to a mass of 1 g.). The terms dekatonie, hectotonie, kilotonie and myriotonie (10,000 tonies) are employed for greater pressures. A myriotonie (*M*) is about one one-hundredth of an atmosphere.[m]

§5. Absorption of Dissolved Substances.—Only a few direct experiments upon the entrance of dissolved substances into the cell are available. Some conclusions concerning the mechanism of absorption may be drawn from plasmolytic experiments with salt solutions. Every substance entering the cell must pass through two membranes, the cell wall and the protoplasmic membrane. Most dissolved substances easily penetrate the cell wall, but the protoplasm is impermeable, or nearly so, to many of these.

The osmotic properties of the protoplasmic membrane are similar to those of Pfeffer's precipitation membranes. Only the living protoplasm is here meant, however; dead protoplasmic membranes have entirely different properties. Thus pigments are persistently retained within the cell sap by the living protoplast, but these and other dissolved substances diffuse out very rapidly after the cell is dead. Like precipitation membranes, the protoplasmic membrane is not completely impermeable to most substances. For example, Pfeffer[3]

[1] **Hamburger, H. J.,** Osmotischer Druck und Ionenlehre in den medicinischen Wissenschaften. 3 v. Wiesbaden, 1902–1904. **Hober, Rudolf,** Physikalische Chemie der Zelle und der Gewebe. 2 Aufl. Leipzig., 1906. [4 Aufl. Leipzig, 1914.] **Brasch, Richard,** Die Anwendung der physikalischen Chemie auf die Physiologie und Pathologie. Wiesbaden, 1901.

[2] **Errera, L.,** Sur la myriotonie comme unité dans les mesures osmotiques. Recueil Inst. Bot. Bruxelles 5: 193–208. 1902.

[3] **Pfeffer, W.,** Ueber Aufnahme von Anilinfarben in lebenden Zellen. Untersuch. Bot. Inst. Tübingen 2: 179–331. 1886–1888.

[l] The degrees of dissociation are actually much greater, however, than are assumed in this discussion. DeVries's isosmotic coefficients are now to be regarded as of historical interest only. The best discussion of the calculation of osmotic values of solutions is that of Washburn, 1921. [See note *e*, p. 109.]—*Ed.*

[m] This unit has never come into general use and it is now highly improbable that it ever will. Pressures are generally stated in terms of millimeters or centimeters of a mercury column or in atmospheres, an atmosphere being 760 cm. of mercury. It seems undesirable to state osmotic pressure in any other terms than those already used for other kinds of pressure.—*Ed.*

succeeded in introducing useless and even injurious substances (such as aniline dyes) into the living cell. He found that the following pigments penetrated: methylene blue, methyl violet, bismarck brown, fuchsin, cyanin, safranin, methyl green, methyl orange, tropæolin OO and rosolic acid. The concentrations of the solutions employed were very low (from 0.001 to 0.00001 per cent.). Some of the dyes, (*e.g.* methylene blue) first enter the cell sap and color it, but form crystals after a time; Fig. 69 shows an alga cell (Zygnema) with crystals formed by methylene blue. Other dyes (*e.g.*, methyl violet) stain the protoplasm itself. In neither case is the cell fatally injured.

Overton[1] studied a number of different dyes and found that the permeability of the protoplasm to these varied according to their chemical constitution. Basic aniline dyes readily enter the cell, but most of their sulphuric acid derivatives penetrate either not at all or very slowly. Dyes that have accumulated in the cells diffuse out when the cells are placed in water, this outward passage being accelerated by the addition of 0.01 per cent. of citric acid to the water.[2] Citric acid thus appears to change the osmotic properties of the protoplasm. No dye accumulates in the cell if the solution contains 0.01 per cent. of citric acid, but the dye is absorbed from the surrounding solution in the absence of the acid. It is thus possible to alter at will the osmotic properties of cells.

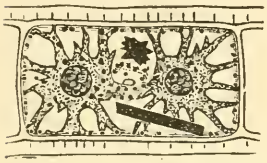

Fig. 69.—Cell of Zygnema with crystals formed by methylene blue.

It is well known that plants can absorb and accumulate the essential chemical elements from very dilute solutions. Some non-essential elements enter the plant cell only until their effective concentration becomes the same within and without, but some others, as well as the essential elements, continue to enter and accumulate in the cell, even from a weak solution, since they are converted into new compounds after entrance and so the internal concentration never becomes equal to the external.

An illustration of continued absorption may be found in the accumulation of iron tannate in an artificial cell of collodion or animal bladder filled with tannin solution and surrounded by one of ferric chloride. Tannin does not escape through the membrane, but ferric chloride diffuses into the cell and there enters into combination with the tannin to form iron tannate, which also remains in the cell. Ferric chloride is continually consumed in the formation of the iron tannate, and its concentration within the cell never becomes the same as that outside. If the tannin solution is sufficiently concentrated

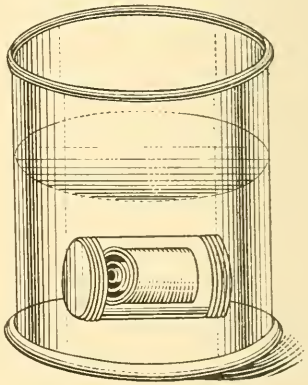

Fig. 70.—Apparatus for showing diffusion of copper sulphate through a membrane into a tube containing zinc.

[1] Overton, E., Studien über die Aufnahme der Anilinfarbe durch die lebende Zelle. Jahrb. wiss. Bot. 34: 669–701. 1900.

[2] Pfeffer, 1886–88. [See note 1, page 121.]

all of the ferric chloride will pass from the outer solution into the cell. In a similar way plant roots appear to absorb the essential elements, as well as other substances, from the surrounding solution.

The following experiment also illustrates this phenomenon of continued absorption (Fig. 70). A roll of sheet zinc is placed in a short glass tube of large diameter, the tube being filled with water and having both ends closed with animal bladder or parchment paper. The tube is placed in a dilute solution of copper sulphate, which passes through the membranes into the tube. Here the copper of the salt is replaced by zinc, and the zinc sulphate thus formed diffuses into the outer solution. Copper sulphate continues to enter until all of it, or all of the zinc, has been used up. The same phenomenon occurs in the growth of bacteria and moulds on various organic compounds. Of two substances having different nutritive values, the cells take up mostly the one with the higher value, frequently leaving the other entirely untouched. For instance, *Aspergillus niger* absorbs only glucose from a mixture of this substance and glycerine, so long as the former is present in the solution.[1]

Outward diffusion through the cell membranes is also subject to regulation. Nathansohn's experiments[2] indicate that sodium chloride easily penetrates the cells of *Codium tomentosum* (a marine alga) but that this salt cannot be completely withdrawn from the cells after it has once entered. When the alga is placed in an isosmotic solution (4 per cent.) of sodium nitrate, the chloride content of the cell sap rapidly decreases at first, but the outward diffusion of chloride ceases after a time, as is clear from the following table. The figures denote chlorine content, calculated as per cent. of HCl.

Original Chlorine Content	Chlorine Content after a Period of				
	1 day	3 days	8 days	15 days	25 days
2.24	0.92	0.93	0.90	0.84	0.76

Plasmolysis of cells has already been described (Fig. 68). DeVries[3] plasmolyzed whole plant organs as well as cells, and showed that growing parts (such as stems, roots and flower stalks) are noticeably shortened after immersion in a plasmolyzing solution, but regain their original stiffness and elasticity when returned to pure water. This rigidity, which is a result of osmostic pressure, is called *turgidity*.

The rate at which water and dissolved substances penetrate the protoplasm

[1] **Pfeffer, W.,** Ueber Election organischer Nährstoffe. Jahrb. wiss. Bot. 28: 206–268. 1895.

[2] **Nathansohn, Alexander,** Zur Lehre vom Stoffaustausch. Ber. Deutsch. Bot Ges. 19: 509–513. 1901.

[3] **Vries, Hugo de,** Untersuchungen über die mechanischen Ursachen der Zellstreckung, ausgehend von der Einwirkung von Salzlösungen auf den Turgor wachsender Pflanzenzellen. Leipzig, 1877. **Idem,** Untersuchungen über die mechanischen Ursachen der Zellstreckung. Halle, 1877.

is influenced by external conditions. Van Rysselberghe[1] studied the effect of temperature upon this rate. In one series of experiments pieces of pith from young twigs of *Sambucus nigra* (elder) were placed in water and then transferred to 26-per cent. solutions of cane sugar at different temperatures. Each piece was 114 mm. in length at the outset, and their lengths were redetermined at stated intervals. The lower the temperature, the more slowly did plasmolysis occur. The amounts of shrinkage observed for such pieces of Sambucus pith, with different temperatures and after different periods of time, are shown in the following table.

Temperature. *deg. C.*	0	6	12	16	20	25
Time Period						
hours	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
2	4.5	8.5	20.0	33.0	40.5	40.5*
4	7.5	13.5	25.0	38.0	42.0*	
6	10.0	17.0	28.0	42.0*		
8	12.5	20.0	30.0			
10	14.0	21.5	31.5			
24	21.0	31.0	40.0			

*No further shrinkage.

In another series of experiments plasmolyzed pieces of Sambucus pith were placed in water at various temperatures, with the same result; the return of turgidity was more rapid as the temperature of the water was higher. These results are shown graphically in the curve of Fig. 71, where the abscissas are the temperatures and the ordinates are the velocities of the movement of water through the protoplasmic membrane (both inward and outward.)

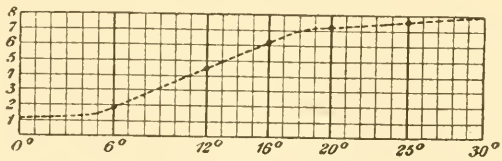

Fig. 71.—Graph representing relation of temperature to velocity of penetration of water through the protoplasmic membrane.

The rates at which dissolved substances diffuse through the protoplasm also depend on temperature. If the velocity of movement at 0°C, be taken as unity, then the following relative velocities are obtained for potassium nitrate, glycerine and urea, for various higher temperatures.

[1] Van Rysselberghe, Fr., Influence de la tempèrature sur la permèabilitè du protoplasme vivant pour l'eau et les substances dissoutes. Recueil Inst. Bot. Bruxelles 5: 209–249. 1902. [Idem, Rèaction osmotique des cellules végètales á la concentration du milieu. Mèm. cour. Acad. Roy. Belgique 58: 1–101. 1898.]

Substance \ Temperature, deg. C.	0	6	12	16	20	25
Potassium nitrate	1.0	1.8	4.4	6.0	7.3	7.3
Glycerine	1.0	1.9	4.2	5.6	7.0	7.0
Urea	1.0	2.1	4.5	5.3	7.0	7.6

The cell sap frequently exhibits high osmotic values.[n] DeVries found that sap expressed from young plant organs showed the osmotic values given in the table below.

Source of Expressed Sap	Osmotic Value
	atmospheres
Gunnera scabra (petioles)	3.5
Solanum tuberosum (leaves)	5.5
Sorbus arcuparia (berries)	9.0
Beta vulgaris (roots)	21.0

The moulds *Aspergillus niger* and *Penicillium* may develop osmotic pressures as great as 157 atmospheres, when they are grown in concentrated sugar or salt solutions.[o]

[n] A solution alone has no osmotic *pressure*, this being produced by two solutions (or a solution and the pure solvent) and a membrane, all acting together. When the "osmotic pressure" of a solution is spoken of, the *maximum* osmotic pressure that might be obtained with that solution, at the given temperature, is meant. To obtain this maximum the membrane employed must be quite impermeable to all the solutes (dissolved substances) of the solution, and the membrane must be in contact with the solution on one side and with the pure solvent (water) on the other. These conditions are probably never actually fulfilled in the case of plant cells. If we employ the term *osmotic value* for the maximum pressure, then the actual pressure developed in any cell is usually of somewhat lower magnitude than is the osmotic value of the cell sap. Diffusion tension of the solute is another term that may be employed for the osmotic value, with reference to the solution itself, but this is not without objection. These measurements of deVries' were made by means of cell membranes (plasmolytic method), so that the nature and condition of the cells used as indicators enter into the argument here, and he was not really measuring the osmotic values of these expressed solutions.—*Ed.*

[o] Fitting has studied the osmotic pressures of the cells of plant leaves, by the plasmolytic method, using potassium nitrate solutions, in a very thorough way. He dealt especially with desert plants. See: **Fitting, Hans,** Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitsch. Bot. **3**: 209–275. 1911. **Livingston, B. E.,** The relation of the osmotic pressure of the cell sap in plants to arid habitats. Plant world **14**: 153–164. 1911. (This is a somewhat critical review of Fitting's paper.) While plant cells in general have osmotic pressures of from 5 to 11 atmospheres, Fitting found pressures much exceeding 100 atmospheres in the leaves of some desert plants. This value is greater for plants growing in

DeVries determined the partial osmotic pressure developed by some of the constituents of the cell sap. The following table gives an idea as to what substances are instrumental in the production of osmotic pressure in plants. The figures denote percentage of the total pressure.

Source of Expressed Sap	Potassium Salts of Organic Acids	Malic Acid	Glucose	Sodium Chloride	Other Substances
Heracleum spondilium (petioles)	5.9	9.1	69.1	6.4	9.5
Rochea falcata (leaves)	3.1	42.3	23.1	11.5	20.0

Diffusion in solution is very important in the absorption of materials by plants but it cannot account for the transfer of absorbed substances within the plant, for movement by diffusion alone is much too slow.[1] For example, it would take 319 days for 1 mg. of sodium chloride, a rapidly diffusing substance, to diffuse 1 m. out of a 10 per cent. solution of that salt. A period of fourteen years would be required for the same amount of albumin to migrate the same distance. Since diffusion progresses rapidly in gelatine and agar as well as in water, these substances may be employed in diffusion experiments, being poured into a glass cylinder while hot and then covered, after cooling, with a solution of the substance to be studied (*e.g.*, indigo). Intercellular protoplasmic connections, like thin threads reaching through the cell walls, are now known to be of common occurrence in plants (Fig. 72). How these structures may influence exchange of materials between the cells is still unknown, however.

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very dry habitats than for those growing in more moist situations. For further studies bearing on this and related matters, see: **Dixon, H. H.,** and **Atkins, W. R. G.,** On osmotic pressures in plants and on a thermo-electric method of determining freezing points. Proc. Roy. Dublin Soc., n.s. **12**: 275–311. 1910. **Idem,** Osmotic pressures in plants. I. Methods of extracting sap from plant organs. *Ibid.* n.s **13**: 422–433. 1913. (Reprinted in: Notes from Bot. Sch., Trinity Coll., Dublin **2**: 154–165. 1913.) **Idem,** same title, II. Cryoscopic and conductivity measurements on some vegetable saps. *Ibid.* n.s. **13**: 434–440. 1913. (Reprinted in: Notes from Bot. Sch., Trinity Coll., Dublin **2**: 166–172. 1913.) **Harris, J. Arthur,** and **Lawrence, John V.,** assisted by **Gortner, R. A.,** The cryoscopic constants of expressed vegetable saps as related to local environmental conditions in the Arizona deserts. Physiol. res. **2**: 1–49. 1916. (Other papers are there referred to.) **Hibbard, R. P.,** and **Harrington, O. E.,** Depression of the freezing-point in triturated plant tissues and the magnitude of this depression as related to soil moisture. *Ibid.* **1**: 441–454. 1916. For a general discussion of the osmotic relations of cells see: **Atkins, W. R. G.,** Some recent researches in plant physiology. xi + 328 p. London, 1916.—*Ed.*

[1] **Stefan, J.,** Ueber die Diffusion der Flüssigkeiten. II. Berechnung der Grahamschen Versuche. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **79**[II]: 161–214. 1879. **Vries, Hugo de,** Ueber die Bedeutung der Circulation und der Rotation des Protoplasma für den Stofftransport in der Pflanze. Bot. Zeitg. **43**: 1–6, 17–26. 1885.

Plants can absorb solid soil constituents but these must first be dissolved in water. If a polished marble plate is placed in the bottom of a box in which seedlings are grown, many of the roots come into close contact with the plate,

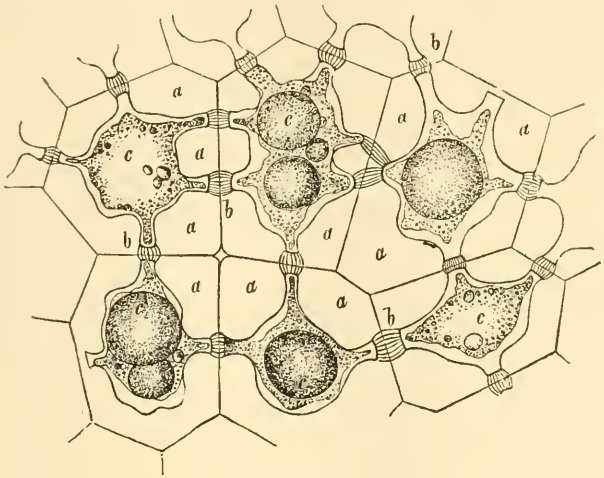

FIG. 72.

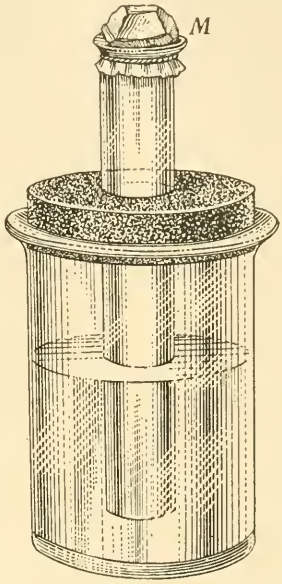

FIG. 73.

FIG. 72.—Cells of endosperm of *Areca oleracea*. *a*, thick cell wall; *b*, canals piercing cell walls and containing protoplasmic strands.

FIG. 73.—A piece of calcium carbonate dissolving in hydrochloric acid as this diffuses upward through the bladder membrane M.

and if the latter is removed after a time the imprint of the roots may be seen on the polished surface, etched by acid root excretion. The acid character of root excretion may also be shown by the reddening of blue litmus paper against which the roots are induced to grow.

The following experiment illustrates the solution of soil particles and their absorption after being dissolved. A broad glass tube with its lower end firmly bound with animal bladder (Fig. 73) is filled with and inverted over a weak solution of hydrochloric acid, so that the cylinder remains filled. A piece of marble is placed upon the smooth surface of the bladder. The marble gradually becomes smaller and smaller as it is dissolved by the acid imbibed in the membrane. Calcium chloride is formed during the process and diffuses slowly through the membrane into the solution below, where it can be identified with suitable chemical reagents.

Czapek[1] studied the nature of root excretions. He employed plates made of a mixture of aluminium phosphate and plaster of Paris. These are soluble in many acids (hydrochloric, nitric, sulphuric, phosphoric, formic, oxalic, succinic, lactic, malic, citric, and tartaric) but they are insoluble in carbonic, acetic, propionic and butyric acids. Various kinds of roots produced no effect

[1] **Czapek, Friedrich,** Zur Lehre von den Wurzelausscheidungen. Jahrb. wiss. Bot. **29**: 321–390. 1896.

upon these plates, when they were exposed to the roots as was the marble mentioned above, and it therefore follows that acids belonging to the first list just given are not noticeably present in root excretions. In other experiments by the same writer Congo red was employed, which becomes brownish-red through the action of carbonic acid and bright blue through the action of acetic, propionic and butyric acid. The roots turned the Congo red only brownish-red, without any tendency toward blue, from which it appears that the corrosion of the marble (in the experiment described above) and of soil particles, is to be attributed to the action of carbonic acid excreted by the roots. According to Stoklasa and Ernest[1] roots excrete organic acids only when inadequately supplied with oxygen.

The following examples indicate how much may be accomplished by plants in dissolving soil particles. Lind[2] showed that the hyphæ of certain fungi in pure culture were able to penetrate through marble plates and bones. Nadson[3] described a considerable number of algæ that penetrate somewhat deeply into limestone and shells, dissolving the material. These forms experience severe competition with many other algæ on the surface of the substratum, but their ability to grow in solid limestone, which is impenetrable to their competitors, gives them a definite advantage in the struggle for existence. Nadson found that these algæ excrete oxalic acid.[p]

It is also well known that parasitic fungi penetrate the cell walls of their host plants. Miyoshi[4] found that fungus hyphæ can pierce membranes of very different kinds. The membranes to be studied were placed over nutrient gelatine and inoculated with spores. As germination took place the hyphæ bored through the membranes and reached the nutrient media below.

Summary

1. **Materials Absorbed by Plants.**—From the air the ordinary plant absorbs carbon dioxide (and also oxygen sometimes, especially at night). From the soil it absorbs water, and inorganic salts that contain nitrogen and the six essential ash constituents (S, P, K, Ca, Mg, Fe). As stated in Chapter III, free nitrogen is absorbed by some lower forms and by the nodule bacteria in the tubercles of legume roots, etc. Small amounts of oxygen appear to be absorbed from the soil by active roots. All these substances, supplying the ten essential elements, and also many that supply non-essential elements, are absorbed by diffusion in solution, generally in aqueous solution. (When the transpiration rate is high, however, it appears that these sub-

[1] **Stoklasa, Julius,** and **Ernest, Adolf,** Beiträge zur Lösung der Frage der chemischen Natur des Wurzelsekretes. Jahrb. wiss. Bot. **46**: 55–102. 1909.

[2] **Lind, K.,** Ueber das Eindringen von Pilzen in Kalkgesteine und Knochen. Jahrb. wiss. Bot. **32**: 603–634. 1898.

[3] **Nadson, G.,** Die perforierenden (kalkbohrenden) Algen und ihre Bedeutung in der Natur. [Abstract in German, pp. 35–40. Text in Russian.] Scripta Botanica Hort. Univ. Imp. St. Petersburg **18**: 1–40. 1900–1902.

[4] **Miyoshi, Manabu,** Die Durchbohrung von Membranen durch Pilzfaden. Jahrb. wiss. Bot. **28**: 269–289. 1895.

[p] Also see: **Diels, L.,** Die Algen-Vegetation der Südtyroler Dolomitenriffe. Ber. Deutsch. Bot. Ges. **32**: 502–526. 1914.—*Ed.*

stances may enter roots from the soil by a mass streaming and filtration of the soil solution through the peripheral cells, to the xylem vessels.) To enter plant cells, these substances must be dissolved in water (or some other substance in the cell wall). They diffuse through the peripheral, water-impregnated cell walls, into the protoplasm. Carbon dioxide and oxygen diffuse through the suberin or lignin of cell walls that are impregnated with one of these substances, as well as through the imbibed water.

2. **Diffusion of Gases.**—The ultimate particles of every gas, and of every mixture of gases, are considered as always in motion (somewhat as the individuals of a swarm of gnats in the air) and as always tending to spread outward in all directions, until some impermeable wall is encountered. They tend to distribute themselves uniformly throughout all the space that is available. This spreading movement of the individual gas particles is called *diffusion* of the gas; it is not to be confused with mass flow and convection, by which the gas streams or flows as a whole, like wind. If two masses of different gases are brought into contact (as in the two halves of a closed chamber) and if no convection or stirring motion is present, the particles of both kinds of gas diffuse outward, each kind into the space of the other kind, as though the other kind were not present, and they eventually become uniformly mixed. Rates of diffusion of different kinds of gases are proportional to the square roots of their respective densities; hydrogen diffuses four times as rapidly as oxygen (densities, 1:16), temperature and pressure being the same for both. If septum or wall separates the two original gas masses, diffusion takes place in both directions through the septum if that is permeable to both gases; if the septum is permeable to but one of the gases, diffusion occurs in one direction only. If the material of the septum is such that the gas dissolves in it, then the gas diffuses through this material in the dissolved state (as a *solute*). Solutes (whether they are gases, liquids, or solids under ordinary conditions) diffuse through the *solvent* in a manner analogous to that of gas diffusion, but the rate of diffusion here is proportional to the concentration gradient in the liquid. With a liquid-water septum separating two different gases (which are at the same pressure and temperature), the rates of diffusion *through the septum* are proportional to the *solubilities* of the two gases in water. There may also be mass streaming of the septum material itself, which would *apparently* alter the rate of this diffusion, the solute being *carried by*, rather than *diffusing in*, the solvent.

3. **Entrance of Gases into Plants.**—Gases, as such, diffuse into (and out of) ordinary plants through stomata and lenticels (openings in the peripheral layer of cells, connecting directly with gas-filled, irregular, intercellular channels in the tissues). Gas diffusion continues in the intercellular spaces. There is also some mass streaming of gases through intercellular spaces and their external openings. But beyond the cell walls bounding these channels gas diffusion and gas streaming do not reach. Through suberized, lignified, or cutinized cell walls, substances that are ordinarily gases diffuse *in solution* in the substance of the walls, as well as in the small amounts of water held by imbibition. Through ordinary, water-impregnated walls (and also through the cell contents) they diffuse as solutes in the water.

Most of the carbon dioxide and oxygen exchange of ordinary plants occurs through the stomata or lenticels, the true absorption (or elimination) occurring, however, at the peripheries of the intercellular channels, where the gases pass into (or out of) solution in the imbibed water of the cell walls that bound these channels. Gas diffusion through stomata occurs at a rate proportional to the *linear* dimensions of the openings or pores, other conditions being constant; the rate is therefore relatively

much greater for these small openings than would be the case if it were proportional to the areas of the cross sections of the openings.

4. **Diffusion of Dissolved Substances.**—Solute particles diffuse outward in the solvent, much as do gas particles in space, and tend to become equally distributed throughout its volume Solute diffusion does not extend beyond the spatial limits of the solvent. Mass streaming or convection accelerates or retards the *apparent* rate of diffusion, just as is true for gases. Solute and solvent particles attract each other. If pure water is separated from an aqueous solution by a septum permeable to both solvent and solute, diffusion of both substances occurs through the septum and a uniform solution on both sides finally results. If the septum is permeable only to the solvent (water), then diffusion takes place only in the direction from solvent to solution, and osmotic pressure is developed in the latter. This is like gas pressure in many respects, being proportional to the outward-diffusing tendency of the solute particles. Salts dissociate or ionize to some extent in solution, and the osmotic pressure that can be developed by a given solution (its osmotic value) is nearly proportional to the total number of particles contained in a unit of volume; more precisely, it is proportional to the quotient of the number of solute particles present divided by the total number of particles (solvent and solute).

When a septum—such as the outer surface of the protoplasm of a cell—separates, two different aqueous solutions, each containing many kinds of solutes as well as water, the septum may retard the diffusion of water or that of any of the solutes, but its presence does not render diffusion any more rapid than it would be if the septum were not present. Retardation may be greater for some substances than for others.

Plasmolysis is the tearing of the protoplasmic lining away from the cell wall, frequently due to the presence of more non-permeating solute particles, per unit of volume, on the *outside* of the protoplasmic periphery than on the *inside*. Turgor results largely from the reverse condition, being generally due to osmotic pressure developed within the cell, by solutes to which the protoplasm is impermeable. This results in the protoplasm being pushed outward against the cell wall, which becomes stretched.

5. **Absorption of Dissolved Substances.**—Most dissolved substances diffuse through cell walls rather rapidly, but the protoplasm is frequently impermeable to many solutes that are present and it retards the inward (or outward) diffusion of others. The permeability of the protoplasm of a cell to the various solutes within and without, alters from time to time, according to conditions in the surroundings and within the cell. Carbon dioxide and oxygen (and other gases in the air) pass into solution in the water, etc., of cell walls and then diffuse as other dissolved substances. These materials, and also salts, etc., dissolved in the soil solution, diffuse through the cell walls and protoplasm of roots. (It appears that they may also be carried in by mass streaming when the transpiration rate is high; if this occurs, the peripheral cells of the roots may act somewhat as filters, allowing the soil water and some of its solutes to enter with the stream but causing other solutes to remain outside or to enter more slowly than do water and the solutes that penetrate the membranes readily.)

A solute may accumulate in the interior of a living cell until its concentration there is higher than that in the solution from which it diffuses. This phenomenon is sometimes to be explained on the ground that the accumulating solute is chemically altered upon passing into the cell, in which case it only *apparently* surpasses the concentration of the solution from which it comes. In other cases the physical explanation is still uncertain. The osmotic value of cell sap is generally between two and six atmos-

pheres, but it may be much higher, as much as 157 atmospheres having been reported for moulds. The actual osmotic pressure in a cell is generally much lower than the osmotic value of its sap.

The rate of diffusion of water and solutes through protoplasm is influenced by temperature, by the condition of the protoplasm, etc., as well as by the concentration difference (or gradient) between the interior and exterior of the cell. Carbon dioxide continually diffuses out of roots into the soil solution (excepting when the transpiration rate is so high that the flow of water into the roots is more rapid than the diffusion rate of carbon dioxide). This substance (forming carbonic acid when dissolved in water) acts as a solvent on many solid soil constituents. Organic acids appear to diffuse out of roots when the latter are poorly supplied with oxygen, and these acids may have a similar action on solid materials in the soil. Some fungi and algæ normally give off organic acids, as do many bacteria also.

CHAPTER VI

MOVEMENT OF MATERIALS IN THE PLANT

§1. **General Occurrence of Movement of Materials.**—From previous statements it is clear that the essential materials are not always directly absorbed by the plant organs in which they are ultimately used. Organic materials are produced from inorganic substances in the green leaf, but the leaf itself can absorb only carbon dioxide. The other materials (water and mineral constituents) that are necessary in the formation of organic compounds are absorbed by the roots, and usually travel long distances before finally reaching the leaves. Similarly, organic materials are frequently used in large quantities in organs where they are not produced; for instance, in all growing parts that lack chlorophyll. This is especially true of organic materials that are elaborated from inorganic compounds; new kinds of organic substances may of course be produced in any region of the plant, from other organic substances that have been previously formed there, or that come from elsewhere. The organic substances that are requisite for the formation of new cells come to these cells from the leaves, and they also frequently travel long distances before reaching the point where they are used, as in the case of growing root-tips. It is clear, therefore, that there is a general movement of materials within the plant.

The compounds occurring in plants may be in the solid as well as in the liquid or gaseous condition. Solid substances, however, must first pass into solution before translocation can occur, since otherwise they cannot pass through cell walls. The study of the movement of materials in plants may, accordingly, be reduced to a consideration of the movement of gases and of water and dissolved substances.

§2. **Movement of Gases.**—Many air passages (intercellular spaces) are always present in the cortex of stems and roots as well as in the parenchymatous tissues of leaves. The lenticels, small openings in the bark, and the stomata also, bring these passages into direct connection with the external air, and the internal atmosphere is thus always under the same pressure as that of the air outside, while renewal of the internal air may readily occur through openings to the outside.

Gas exchange through the cortex of water plants is greatly hastened by differential diffusion of air, which was first observed in the leaves of *Nelumbium speciosum*.[1] The leaf of this plant consists of a round leaf-blade, from the center of the lower surface of which the petiole projects. Stomata occur only

[1] **Barthélemy, A.**, De la respiration et de la circulation des gaz dans les végétaux. Ann. sci. nat. Bot. V. 19: 131–175. 1874. [See also, for observations and a better explanation: **Ohno, N.**, Ueber lebhafte Gasausscheidung aus den Blättern von Nelumbo nucifera. Zeitschr. Bot. 2: 641–664. 1910. [Rev. by **Livingston** in: Plant world 14: 72–73. 1911.]

on the upper surface. If water happens to lie upon the upper leaf surface gas bubbles are observed to be given off rapidly on sunny days, these bubbles arising from the stomata and from any chance openings in the surface of the petiole. This evolution of gas is so violent at times that the water appears to be boiling. This phenomenon is unrelated to life processes, since it occurs also with dead leaves. A similar elimination of gas may be artificially produced by a special arrangement. This consists of a cylindrical porous clay cell filled with finely powdered chalk, or simply with air. A glass tube is inserted through a stopper closing the open end; this tube corresponds to the petiole of the Nelumbium leaf, while the cell corresponds to the leaf-blade. The porous cell is first dipped in water and is then supported obliquely, the tube ending in a vessel of water below. When the clay cell is heated, gas is given out in large quantities from the open end of the glass tube. This gas is air, practically saturated with water vapor. Frequently the volume of gas thus eliminated is as much as forty times as great as that of the cell itself, so that gas must enter the cell through the porous wall during the experiment. This phenomenon is caused by unequal heating, both in the case of the porous clay cell and in that of the Nelumbium leaf.[a]

The underground portions of many plants growing in submerged, swampy, or poorly aerated soils,[b] possess root outgrowths that grow upward into the air

[a] Ohno found the pressure under which gas escapes from Nelumbo leaves to rise sometimes to more than 40 mm. of a mercury column. The explanation is somewhat complicated. The gas pressure outside the clay chamber is due to a large partial pressure of oxygen and nitrogen and a very much smaller one of water vapor, the magnitude of the latter depending upon the humidity of the air. The conditions are reversed on the inside, where the larger partial pressure is due to water vapor and that due to the other gases of the air is smaller. The wet porous clay wall, being permeable to the other gases as well as water, movement takes place in both directions; water moves outward and evaporates, and nitrogen and oxygen diffuse inward. Since there is an excess of liquid water, the partial pressure of water vapor on the inside remains constant in spite of the outward movement. Also, the water vapor that evaporates from the external surface of the porous clay is quickly removed from the vicinity by air currents, so that the partial gas pressure due to water vapor on the outside also remains nearly constant. The external partial pressure of nitrogen and oxygen is also constant, in spite of the inward diffusion, for there is here an excess of these gases and the whole atmosphere is available. But, as these gases diffuse into the chamber they raise the partial pressure of non-aqueous gases within, and so increase the total gas pressure on the inside. Since the chamber opens to the outside through the tube, this internal gas pressure can never rise much above what it was at the start, for bubbles escape from the open end of the tube. The arrangement is a sort of osmometer, with a concentrated solution of water vapor in the other gases on the inside and a very dilute solution of the same sort on the outside, the wet wall being more permeable to nitrogen and oxygen than to water vapor. A relatively large amount of water vapor is contained in the gas that exudes from the tube. The heating of the tube seems to accelerate the process partly because it tends to remove the water vapor as it evaporates from the tube, so as to keep the external partial pressure of the other air gases near its original high value. It thus acts like a stirrer in an osmometer cell, which keeps the internal solution from becoming too much diluted next to the membrane. Also, at higher temperature the vapor pressure of water inside the chamber is higher.—*Ed.*

[b] These structures (called "knees") are characteristic of *Taxodium distichum* (bald cypress), of the swamps of the southeastern United States. For an excellent photograph showing these see: **Schimper-Fisher,** 1903. [See note *k*, p. 101.] Fig. 48, facing p. 74.—*Ed.*

(Fig. 74). The tips of these are composed of spongy tissue and readily allow the entrance of air. These outgrowths are like ventilation pipes in that they promote the movement of air to the roots below. The air spaces of the cortex are thus always directly or indirectly in communication with the external atmosphere.[1]

The central woody cylinder of the stem also contains air. Höhnel's[2] experiments indicate that the air in the wood and that in the cortex are not at all continuous. In these experiments (Fig. 75) a leaf is fastened, by means of a rubber stopper, in a wide-mouth bottle (*g*), which has a lateral opening below, the latter fitted with a tube and funnel (*J*) through which mercury is introduced. The air in the cylinder, compressed by the mercury, is forced through the suspended leaf and rises in bubbles through the water in the glass vessel above (*f*).

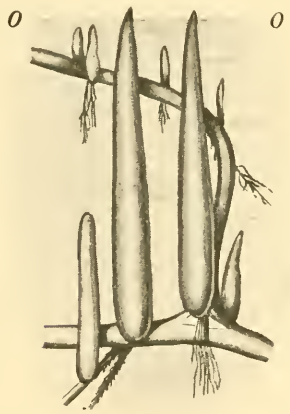

FIG. 74.—Part of stem of *Jussiæa repens*, with ventilation roots (*w*); surface of water, *O*.

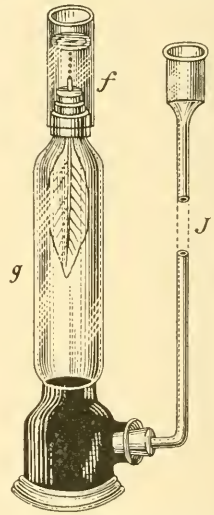

FIG. 75.—Höhnel's apparatus. (*After Pfeffer.*)

Microscopic study shows that bubbles are extruded only from the cortex, not from the central cylinder. Air enters the leaf through the stomata and air spaces of the leaf cortex and is given out from the cortical region of the stem, without entering the wood. The aeration system of the wood is a closed system and does not communicate at all with that of the cortex.

Höhnel demonstrated the existence of *negative gas pressure* in the wood of stems. If a twig, or the petiole of a leaf, is cut under mercury on a sunny day in summer, mercury rises very rapidly through the cut surface into the vessels (Fig. 76), which then appear gray in cross-section, from the presence of the

[1] Goebel, K., Ueber die Luftwurzeln von Sonneratia. Ber. Deutsch. Bot. Ges. 4: 249–255. 1886. Jost, Ludwig, Ein Beitrag zur Kenntniss der Athmungsorgane der Pflanzen. Bot. Zeitg. 45: 601–606, 617–627, 633–642. 1887.

[2] Höhnel, Franz Xavier R. von, Einige anatomische Bemerkungen über das räumliche Verhältniss der Intercellularräume zu den Gefässen. Bot. Zeitg. 37: 541–545. 1879. Idem, Beiträge zur Kenntniss der Luft- und Saftbewegung in der Pflanze. Jahrb. wiss. Bot. 12: 47–131. 1879–1881.

metal. Occasionally vessels may thus be injected with mercury for a distance of from 50 to 60 cm. above the cut surface. Experiments of this kind show that the attenuation of the air in the vessels may be very considerable. Negative pressure in wood may be demonstrated in still another way. A leafy branch with two or more twigs (Fig. 77) is placed with its cut end in water. One of the twigs is cut off and the cut end (*b*) is connected with rubber tubing to a glass tube (*a*), the lower end of which dips into mercury. After some time the mercury rises in the tube indicating that the air in the wood is rarefied. The air of the stem is most attenuated when the activity of the plant is greatest.[c] As will be seen later, this phenomenon of negative gas pressure bears an important relation to the movement of water in the stem.

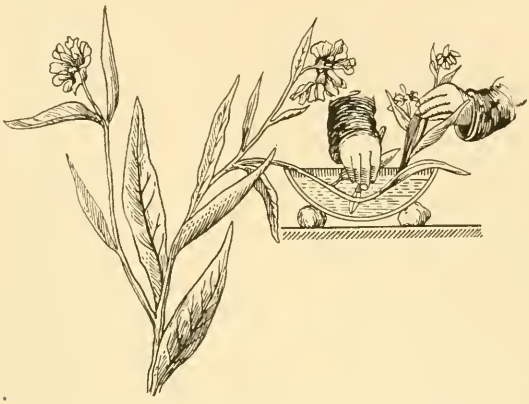

FIG. 76.—The cutting of a stem under mercury.

§3. **Movement of Water and Dissolved Substances.**—The first experiments upon the movement of water and solutes in plants were carried out by Malpighi in 1671. He removed a ring of bark from a woody stem and found that the region above the wound continued alive and grew even more rapidly than before, producing an annular swelling (Fig. 78), while the region below the wound failed to develop further. The girdling operation is thus seen to have no effect at all upon the movement of water from the soil into the upper portion of the plant, although it stops the movement of organic materials into the lower regions. Malpighi concluded from this experiment that the soil solution moves upward through the wood, while the organic substances produced in the leaves pass downward through the cortex. The movement of water is sometimes spoken of as the ascending current, and that of organic (or plastic) substances as the descending current. The expressions ascending and descending are not to be interpreted literally, however; in the drooping branches of the weeping willow, for example, the ascending stream *descends* and the descending one

[c] The phenomenon is mainly dependent upon the rate of loss of water by transpiration from the leaves and upon the rate at which water reaches the leaves from below. The word activity, as used in the text, is rather indefinite, but it may be taken to refer to conditions promoting high transpiration rates.—*Ed.*

ascends. If a ring of bark is removed from a drooping branch of this willow, the swelling develops not above but below the wound.

§4. **The Transpiration Stream.**—The upward movement of the soil solution in the plant depends upon a large number of conditions. Water can enter the plant only if a part of the water already present be lost.[d] Water is removed from the plant by evaporation from the leaves, the process being called transpiration, and this is the main condition determining the movement of water.

(*a*) *Transpiration.*[e]—Transpiration may be studied in a number of ways, some of which will now receive attention.

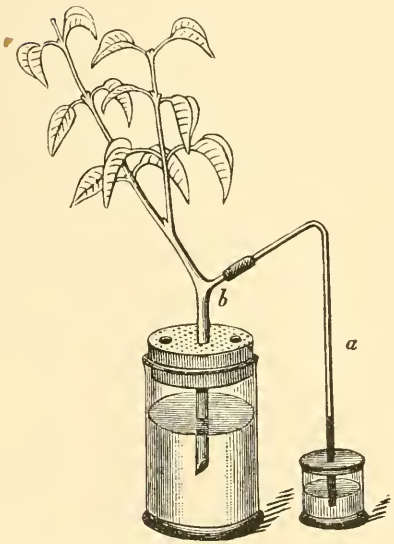

FIG. 77.—Apparatus for showing negative gas pressure in wood. (*After Pfeffer.*)

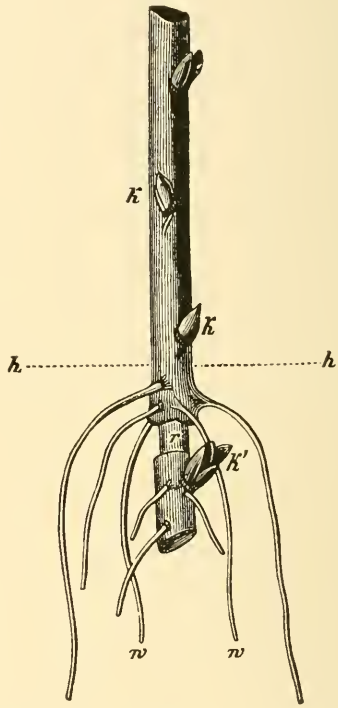

FIG. 78.—Malpighi's girdling experiment; the twig is immersed in water to the line *h*.

1. The quantity of water transpired may be found by determining the loss in weight of the plant and its container. The pot in which the plant is rooted is hermetically sealed in a sheet-metal container. The seal (which may be of plastiline or of a mixture of paraffine and petrolatum, etc.) should have three

[d] While this is the main consideration, it may be remembered that enlargement alone, without any loss of water, must necessitate water entrance into the enlarging cells. Also, water may be removed from a cell and still not pass out of it, as when it becomes chemically combined within (formation of carbohydrate from water and carbon dioxide, formation of glucose from starch, etc.).—*Ed.*

[e] For an excellent review of the literature of transpiration, see: **Burgerstein, A.,** Die Transpiration der Pflanzen. Jena, 1904. Also: Zweiter Teil (Ergänzungsband). Jena, 1920.—*Ed.*

openings, through one of which the stem of the plant projects. The second opening is usually closed and bears a tube through which water may be added to the pot, and the third bears a small glass tube drawn to a fine, open point above. Through the capillary opening of this tube the air in the apparatus remains in equilibrium with that of the external atmosphere. The loss in weight of the apparatus is due almost entirely to the loss of water from the plant by evaporation.[1] A tall cylindrical vessel of water may be used for small plants in experiments of short duration. The plants are fastened, by means of silk-wrapped wire, with their roots in the water and their green parts projecting into the air, a thin layer of oil being placed over the water surface to prevent evaporation.[f] The loss in weight of the apparatus, in this case also, is due almost

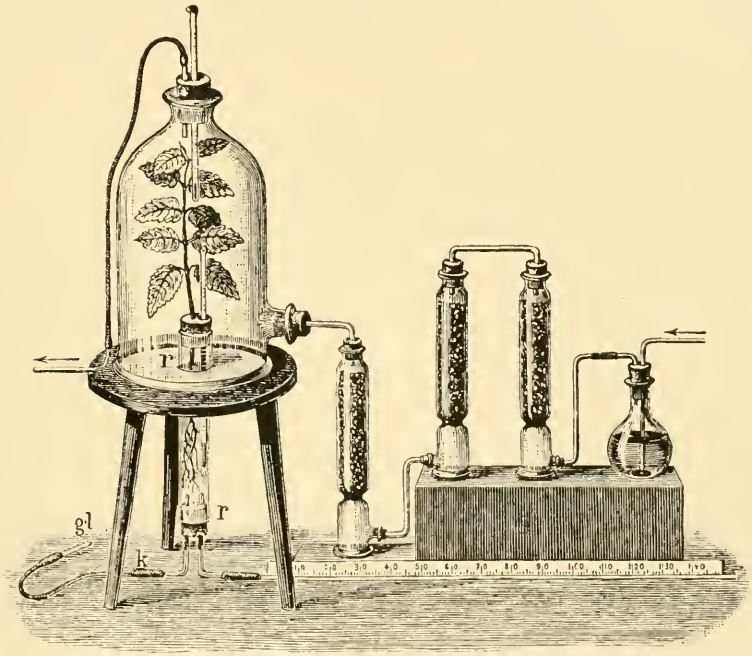

FIG. 79.—Kohl's apparatus for the study of plant transpiration.

wholly to evaporation of water from the plant.[2]

2. The amount of water absorbed by the plant may be measured, Kohl's[3]

[1] **Hales, Stephen,** Vegetable Staticks. London, 1727.

[2] **Wiesner, Julius,** Untersuchungen uber den Einfluss des Lichtes und der strahlenden Wärme auf die Transspiration der Pflanze. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien 74[1]: 477–531. 1877.

[3] **Kohl, F. G.,** Die Transpiration der Pflanzen and ihre Einwirkung auf die Ausbildung pflanzlicher Gewebe. Braunschweig, 1886.

[f] Oil is apt to penetrate into the stem, and the wax seal is much to be preferred. For a short distance above and below the water surface, the stem may be covered with some material (as plastiline, chicle—the base of the common chewing-gum of the American market—etc.) that does not absorb water and prevents the oil from coming into contact with the plant, in which case the oil-seal method may be satisfactory. Some of the plastiline on the American market is unsuitable, however, for it injures some plants.—*Ed.*

apparatus, shown in Fig. 79, being well suited to such studies. The roots of the plant, together with a thermometer, are placed in a tube of water (*r*), which communicates below with a long capillary glass tube and also with a rubber tube closed with a glass plug (*gl*). As transpiration proceeds, the water meniscus advances along the capillary tube. To refill the latter, the glass plug is simply inserted somewhat farther into the rubber tube. By placing a bell-jar over the plant the atmosphere surrounding the latter may be kept either moist or dry. To keep it moist a sponge saturated with water may be placed under the bell-jar, the walls of which may also be moistened. To keep the atmosphere dry, air may be drawn by an aspirator through a series of wash bottles filled with concentrated sulphuric acid or with pieces of pumice saturated with this acid. The plant may be kept in darkness by covering the bell-jar with an opaque paper cylinder.

3. Finally, the amount of liquid water absorbed and the amount of water vapor lost at the same time may be determined. In this connection, Vesque's[1] apparatus may be used, which consists of a U-shaped tube, one arm of which is broad and the other narrow. This is filled with water and the roots of the plant are placed in the broad arm with a tightly fitting stopper about the stem. Loss in weight of the entire apparatus gives the quantity of water evaporated, while the depression of the water in the narrow arm indicates the amount of water absorbed by the plant.[g]

In addition to the apparatus already described, cobalt paper was employed by Stahl[2] to study transpiration. Swedish filter paper is dipped in a 5-per cent. solution[3] of cobalt chloride, and is then dried in the sun or in an oven. It should be stored in a dry place. This paper is intensely blue when dry but the color changes to a bright pink as water is absorbed. The paper is placed upon the leaf surface that is to be studied, and is covered with a small glass or mica plate. For example, a slip of dry cobalt paper, placed against the lower surface of a leaf with stomata on this side only, turns pink in a few seconds on a sunny day, but may remain blue for several hours when placed against the upper leaf surface, where stomata are lacking. This experiment shows clearly the influence of stomata upon transpiration.[h]

[1] **Vesque, Julien,** L'Absorption comparée directement à la transpiration. Ann. sci. nat. Bot. *VI*, 6: 201–222. 1877.

[2] **Stahl,** 1894. [See note 1, p. 36.]

[3] Weaker solutions (1- or 2-per cent.) are more suitable in delicate tests, where the differences in transpiration are small.

[g] It is not strictly true that loss of weight in these experiments is to be interpreted solely as loss of water, though other losses are generally negligible. Perhaps the only case where significant errors may be involved on account of this assumption is that in which leaves, etc., fall from the plant during an experiment. For a complete picture of the meaning of loss of weight, however, aside from such obvious accidents as the fall of leaves, it should be remembered that carbon dioxide and oxygen leave the plant in the same way as does water vapor, that absorption of these two gases also occurs, and that many volatile oils, etc., also evaporate into the air to some extent.—*Ed.*

[h] The cobalt-chloride method really furnishes a means for measuring only the power of the leaf to retard water loss by transpiration, the transpiration rate itself depending upon the evaporating power of the air and upon the intensity of absorbed radiant energy as well as upon this power. On various improvements upon Stahl's method and upon the transpiring

The amount of water lost from plants by evaporation is very large; in Wiesner's experiments, for instance, three maize seedlings weighing 1.6 g. lost 0.198 g. of water during a single hour in sunlight. Wollny[1] measured the amount of water lost by evaporation from several plants during their entire vegetative period and also determined the dry weights of the harvested plants and the amounts of water evaporated for each gram of dry material for the entire period of growth.[i] These values, in grams, appear in the table below.

Kind of Plant	Loss from Plants and Soil Together						Total Loss For Whole Period		Plant Loss, per Gram of Dry Weight
	June	July	Aug.	Sept.	Oct.	Total	From Soil	From Plants	
									grams
Maize	647	3113	5761	2754	...	12,275	1063	11,212	233
Oats	482	2095	2733	2008	...	7,318	178	7,140	665
Pea	773	978	917	941	801	4,410	234	4,176	416

Although plants evaporate large amounts of water, as is evident from the data just given, the amount of water lost from a certain area of leaf is considerably less than that lost from an equal area of a free water surface. According to Hartig, 1 sq. m. of free water surface lost 2000 cc. of water in twenty-four hours, while an equal area of beech leaves lost only 210 cc.[j]

power of leaves, see: **Livingston, B. E.,** The resistance offered by leaves to transpirational water loss. Plant world **16**: 1–35. 1913. **Bakke, A. L.,** Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. Jour. ecol. **2**: 145–173. 1914. **Livingston, B. E.,** and **Shreve, Edith B.,** Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant world **19**: 287–309. 1916.—*Ed.*

[1] **Sachsse, Robert,** Lehrbuch der Agriculturchemie. Leipzig, 1888. P. 423. [**Whollny, E.,** Der Einfluss der Pflanzendecke und der Beschattung auf die physikalischen Eigenschaften und die Fruchtbarkeit des Bodens. 197 p. Berlin, 1877. P. 126.]

[i] This ratio has been called the *water requirement.* For an excellent review of the literature of this subject see: **Briggs, L. J.,** and **Shantz, H. L.,** The water requirement of plants. II. A review of the literature. U. S. Dept. Agric., Bur. Plant Ind., Bull. 285. 1913.—*Ed.*

[j] Such comparisons are without very much significance unless the two surfaces that are compared have the same shape and the same exposure. In such studies as that here referred to it has frequently been the practice to compare evaporation rates from circular, horizontally exposed, free water surfaces with the corresponding rates of transpiration from an equivalent area of plant leaves. The form and exposure of the latter surface is generally exceedingly complex, while these characters of the water surface are relatively simple, and no very useful comparison is possible by such methods. The evaporating surface of the physical apparatus must resemble the plant surface, in form, size, color, etc., as nearly as is practicable. In this connection, see **Renner, O.,** Experimentelle Beiträge zud Kenntnis der Wasserbewegung. Flora **103**: 171–247. 1911. **Idem,** Zur Physik der Transpiration. Ber. Deutsch. Bot. Ges. **29**: 125–132. 1911. **Idem,** Zur Physik der Transpiration II. *Ibid.* **30**: 572–575. 1912. Perhaps the Livingston spherical porous-cup atmometer furnishes the best evaporating surface for comparison with plants in general, but for detailed study a special atmometer constructed after the pattern of the particular plant used should be employed. On the porous-cup atmometer see: **Livingston, B. E.,** Atmometry and the porous-cup atmometer. Plant world **18**: 21–30, 51–74, 95–111, 143–149. 1915. **Livingston, B. E.** and **Thone, Frank,** A simple non-absorbing mounting for porous porcelain atmometers. Science, n. s. **52**: 85–86. 1920.—*Ed.*

Leaves removed from the plant lose much more water than those still attached to the plant. Krutizky[1] found that a single leaf of *Cyssus antarcticus* lost 10.6 cc. of water in one day, while a branch of the same plant with six leaves, lost only 10.8 cc. Results obtained from studies with cut leaves are thus not to be applied directly to entire plants.

After the foregoing introductory remarks, the *influence of external conditions upon the rate of transpiration* will now be considered.

Light exerts a pronounced influence upon the amount of water evaporated.[2] For instance, three maize seedlings weighing 1.6 g. transpired in one day, 198 mg. in sunlight, 68 mg. in diffuse light and 27 mg. in darkness. Plants transpire much more actively in light than in darkness. If they are transferred from darkness to light, or the reverse, the rate of transpiration is not suddenly increased or decreased, but the change in rate takes place gradually.

The daily periodicity of transpiration also depends upon light.[3] The amount of water absorbed during the whole period of twenty-four hours is practically equal to that lost by transpiration in the same period, but there is no such agreement between the rates of absorption and transpiration for the various hours of the day; plants are generally nearly saturated with water at night but during the day there is a saturation deficit.[k]

All rays of the spectrum are not equally effective in promoting transpiration from green plants, the maximum effect is produced in the blue and violet regions. The red rays between the Fraunhofer lines B and C are next, in order of their influence. The same wave-lengths of light that are most absorbed by chlorophyll are thus also most effective in promoting transpiration.

Of all the external factors influencing transpiration, light is undoubtedly the most important. The question arises as to how much light is used in this process. An experiment[4] showed that sunflower leaves transpired on a sunny day 275 cc. per square meter of leaf surface per hour. To evaporate this amount of water requires 166,800 gram-calories of heat per hour. This leaf area received 600,000 calories per hour, so that it appears that 27.5 per cent. of the total amount of radiant energy received was used up in transpiration; as will be remembered, only about 0.5 per cent. is used up in the assimilation of carbon.

[1] **Famintsyn, A.,** Exchange of materials and transformation of energy in plants. [Russian.] Zapiski Akad. Sci. St. Petersburg **46,** Appendix. xvi+816 p. 1883.

[2] **Baranetsky, J.,** Ueber den Einfluss einiger Bedingungen auf die Transpiration der Pflanzen. Bot. Zeitg. 30: 65–73, 81b–89b, 97–109. 1872. **Wiesner,** 1877. [See note 2, p. 135.] **Kohl,** 1886. [See note 3, p. 135.]

[3] **Eberdt, O.,** Die Transpiration der Pflazen und ihre Abhängigkeit von äusseren Bedingungen. Marburg, 1889.

[4] **Brown** and **Escombe,** 1900. [See note 1, p. 34.]

[k] **Renner, O.,** Beiträge zur Physik der Transpiration. Flora **100**: 451–547. 1910. **Idem** Versuche zur Mechanik der Wasserversorgung. 1. Der Druck in den Leitungsbahnen von Freilandpflanzen. Ber. Deutsch. Bot. Ges. **30**: 576–580. 1912. **Idem,** same title. 2. Ueber Wurzeltätigkeit. *Ibid.* **30**: 642–648. 1912. **Livingston, B. E.,** and **Brown, W. H.,** Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. gaz. **53**: 309–330. 1912. **Lloyd, F. E.,** Leaf water and stomatal movement in Gossypium and a method of direct visual observation of stomata *in situ.* Bull. Torrey Bot. Club. **40**: 1–26. 1913. **Shreve, Edith B.,** The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Pub. 194. 1914.—*Ed.*

Although leaves removed from the plant evaporate much more water than do apparently similar attached leaves, nevertheless this experiment shows that considerably more solar energy disappears in the process of transpiration than in the decomposition of carbon dioxide.

The humidity of the surrounding air is a second condition markedly influencing the rate of transpiration. The less water vapor the air contains, the more rapid is transpiration, and the transpiration rate decreases as the water-vapor content of the air increases.[l]

Temperature also influences transpiration, but the relation here is complicated by the fact that life-processes in general are greatly affected by temperature.

Movement of the air also increases transpiration. Finally, the chemical properties of the soil exert a marked influence upon the amount of water evaporated from leaves. Experiments with water cultures show that transpiration is controlled both by the concentration of the solution and by the presence or absence of certain substances. Thus, acids may accelerate, while alkalies may retard transpiration. Addition of a small amount of some salt to distilled water in which plants are rooted produces an increased rate of transpiration, but addition of larger amounts causes a gradual decrease in the rate. The transpiration of plants grown in solution containing the essential mineral elements becomes less as the concentration of the solution is increased.[m]

Besides the external factors mentioned above, there are also internal conditions that control transpiration, these being related to the organization of the plant. First, the age of the plant is important. During the period of greatest activity of the leaf, while it is still growing, the rate of transpiration is highest. The reason for this is that the epidermis of young leaves is very permeable to water; transpiration decreases later, but a second maximum is reached when the stomata begin to function. Thereafter the rate of transpiration gradually decreases as the epidermis hardens, in spite of the influence of the stomata.

The rate of transpirational water loss from leaves is also correlated with the form and character of their anatomical structures (*e.g.*, number of stomata, thickness or permeability of the epidermis, etc.). A discussion of the resistance offered by plants to transpiration will be presented later, in Part II, Chapter III.

Liquid water, as well as water vapor, is given out from many plants, through *hydathodes*.[n] The exudation of liquid water may partly replace transpiration,

[l] The best study of the influence of air humidity as such is: **Darwin, F.**, On a method of studying transpiration. Proc. Roy. Soc. London **B87**: 269–280. 1914. Reviewed by **Livingston** in: Plant world **17**: 216–219. 1914.—*Ed.*

[m] On the influence of chemicals upon transpiration see: **Reed, Howard S.**, The effect of certain chemical agents upon the transpiration and growth of wheat seedlings. Bot. gaz. **49**: 81–109. 1910. On the influence of the osmotic concentration of the medium see: **Briggs** and **Shantz**, 1913 [see note *i*, p. 137]; **Tottingham**, 1914, [see note *d*, p. 84]; **Shive**, 1915, 2 [see note *a*, p. 83]; **Trelease**, 1920, [see note *e*, p. 86].—*Ed.*

[n] **Moll, J. W.**, Ueber Tropfenausscheidung und Injection bei Blättern. Bot. Zeitg. **38**: 49–54. 1880. **Idem**, Untersuchungen über Tropfenausscheidung und Injection von Blättern. Verslag. en Meded. K. Akad. Wettensch. Naturk. Amsterdam 2 R., **15**: 237–337.

occurring mostly when transpiration is retarded for some reason, as for example, in the case of the Aroideæ and other plants living in moist places (Fig. 80).°

(*b*) *Exudation Pressure.*—The second condition determining the movement of water in stems is the so-called root pressure, sap pressure, or exudation pressure, which produces bleeding. This phenomenon was first investigated by Hales.[1] If a branch is cut from a grapevine in the spring, before the buds open, a watery fluid is extruded from the wound. Hales bound a piece of animal bladder over the cut end and found that the sap was excreted with such force that the bladder was much swollen at first and was finally broken. To measure the force with which the sap was extruded, Hales connected the cut end of a branch with

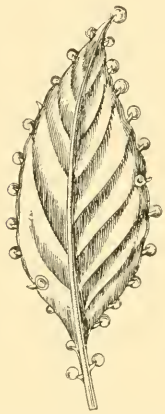

FIG. 80.—Guttation from hydathodes at the edge of a leaf of *Impatiens sultani*. (*After Pfeffer.*)

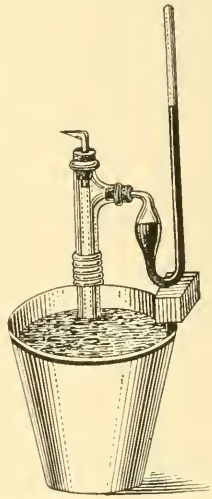

FIG. 81.—Arrangement for measuring exudation pressure. (*After Pfeffer.*)

1880. **Volkens, G.**, Ueber Wasserausscheidung in liquider Form an den Blättern höherer Pflanzen. Jahrb. K. Bot. Gart. u. Bot. Mus. Berlin **2**: 166–209. 1883. **Gardiner, Walter,** On the physiological significance of waterglands and nectaries. Proc. Cambridge Phil. Soc. **5**: 35–50. 1883. **Wieler, A.**, Das Bluten der Pflanzen. Cohn's Beiträge zur Biol. d. Pflanzen **6**: 1–211. 1893. **Haberlandt, G.**, Anatomisch-physiologische Untersuchungen über das tropische Laubblatt. II. Ueber wassersecernirende und-absorbirende Organe. (I. Abhandlung.) Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **103**[I]. 489–538. 1894. **Idem,** same title (II. Abhandlung.) *Ibid.* **104**[I]: 55–116. 1895. **Idem,** Zur Kenntniss der Hydathoden. Jahrb. wiss. Bot. **30**: 511–528. 1897.—*Ed.*

[1] **Hales,** 1735. [See note 1, p. 135.]

° *Guttation*, as Burgerstein terms this excretion of liquid water [see note *e*, p. 134], may be induced in many plants by injecting the cut stem or petiole with water under pressure. A simple way is to attach a cut branch, by a rubber tube (properly reinforced with cloth wrapping), to the water-tap, having first filled the tube with water, and then to open the tap. Fuchsia, *Impatiens sultani*, and *Tropæolum majus* (garden nasturtium) serve very well. This phenomenon was first described by **deBary** (Bot. Zeitg. **27**: 883. 1869). See also, for another early description: **Prantl, K.**, Die Ergebnisse der neueren Untersuchungen über die Spaltöffnungen. Flora **55**: 305–312, 321–328, 337–346, 369–382. 1872.—*Ed.*

a mercury manometer (Fig. 81). The mercury is forced up in the free arm of the tube by the pressure of the exuding sap, attaining a height, in one of Hales' experiments, of 103 cm. or about 1.5 atmospheres. Instead of removing a branch, an incision may be made in the stem. Bleeding is characteristic of many woody plants in the spring; this is called spring bleeding, since it occurs only in the spring before the leaves expand. After the leaves expand an incision in the stem or the removal of a branch usually fails to produce bleeding; water is then being lost from the leaves by transpiration. Under these conditions bleeding may be induced at the surface of the stump of a cut stem, the leafy portion having been entirely removed. Bleeding may be demonstrated in this way throughout the entire vegetative period, in both woody and herbaceous plants, but the same plant may not show it at all times during its period.

To measure the *force* with which sap is extruded, a mercury manometer is connected to the cut stump of the plant. To measure the *amount* of liquid excreted the manometer may be replaced by a glass tube connecting with a graduate. The recording apparatus of Baranetskii serves the same purpose. Here the liquid flows into a U-shaped tube, lifting a float in the free arm. The float is fastened to one end of a silk thread that passes over a pulley, and a pointer attached to the other end of the thread traces a curve on a smoked, rotating drum. In another apparatus constructed by Baranetskii, the excreted liquid is caught in separate tubes, each tube remaining beneath the outlet tube from the plant for a single hour. The tubes are arranged on the rim of a wooden disk with vertical axis, and this is rotated, by clockwork, just far enough every hour to place a fresh tube under the outlet.

Exudation pressure, as indicated by the height of a mercury column, varies in different plants, being less in herbaceous than in woody forms. Thus, in Hofmeister's[1] experiments the height attained by the mercury column was 66 mm. with *Atriplex hortensis*, and 461 mm. with *Digitalis media*.

The amount of sap excreted by herbaceous plants greatly exceeds the total volume of their roots. Much of the excreted liquid must therefore enter the roots after the cut is made. A plant of *Urtica urens* excreted 3025 cc. of sap, and the total volume of its root system proved to be only 1350 cc. Similarly, the root volume of a plant of *Helianthus annuus* was only 3370 cc., and yet this plant excreted from its cut stump 5830 cc. of liquid.[p]

There is a daily periodicity in the rate of bleeding[2] and this has no relation to temperature. The time of occurrence of the maximum and of the minimum rate of liquid excretion is not the same for different plants. Etiolated plants exhibit no periodicity. Analyses of the sap extruded by bleeding stems are

[1] **Hofmeister, W.,** Ueber das Steigen des Saftes der Pflanzen. Flora, n. R. 16: 1–12. 1858. **Idem,** Ueber Spannung, Ausflussmenge und Ausflussgeschwindigkeit von Säften lebender Pflanzen. *Ibid.* n. R. 20: 97–108. 1862.

[2] **Baranetzky, J.,** Untersuchungen über die Periodicität des Blutens der Krautigen Pflanzen und deren Ursachen. (Besonders abgedruckt aus den Abhandl. Naturf. Ges. Halle 13.[I]) 63 p. Halle, 1873.

[p] In this connection see: **Hofmeister, W.,** Ueber Spannung, Ausflussmenge und ausflussgeschwindigkeit von Säften lebender Pflanzen. Flora **45**: 97–108, 113–120, 138–144, 145–152, 170–175. 1862. The numbers given in the text are from this paper.—*Ed.*

very interesting. In Ulbricht's[1] experiments, potato tubers planted on April 11, produced stems that bloomed on July 5. On July 9 the stems were cut off at from 4 to 6 cm. above the soil. The sap that exuded during the next five days was collected, the exudation for each day being kept separate, so that five portions of sap were available for analysis, the results of which are shown in the following table. The quantities (stated in milligrams) refer to a liter of sap in all cases.

	First Day	Second Day	Third Day	Fourth Day	Fifth Day
Combustible material	450	310	220	280	295
Ash	1160	980	960	910	945
Total dry weight	1610	1290	1180	1190	1240

These numbers show plainly that the total solids consisted mainly of mineral substances, but this statement is still further emphasized by the fact that the combustible material does not represent organic matter alone, for nitric acid and some other inorganic substances are vaporized during incineration, so that it is certain that the sap always contained more inorganic substances than the data show. This result was to be expected, since the ascending water current distributes absorbed soil solution throughout the plant. The presence of organic substances in sap extruded from the xylem may be explained by the fact that the soil solution does not enter this tissue directly, but is transferred into the wood soon after its entrance. It can hardly be supposed that parenchymatous cells, which are so rich in organic substances, should excrete nothing but inorganic materials into the vessels.

The composition of sap excreted in early spring is very different from that of sap excreted in summer. Birch sap was collected from an opening in the tree trunk just above the soil surface, on each of six different days, between April 5 and May 22.[2] The sugar, protein, malic acid, and ash contents per liter of sap are given below, in milligrams, together with the dates on which the sap

Date of Flow	Sugar	Protein	Malic Acid	Ash
April 5	12,500	..	...	....
April 11	13,500	.	332	500
April 17	10,900	21	...	640
May 2	10,100	6	...	1080
May 19	9,400	6	437	....
May 22	6,900	..	...	..

[1] **Ulbricht, R.,** Ein Beitrag zur Kenntniss der Blutungssäfte einjahriger Pflanzen. Landw. Versuchsst. 6: 468–474. 1864. [**Idem,** same title. *Ibid.* 7: 185–192. 1865.]

[2] **Schroeder, Julius,** Die Frühjahrsperiode der Birke (*Betula alba* L.) und des Ahorn (*Acer platanoides* L.) Landw. Versuchsst. 14: 118–146. 1871.

was collected. It is apparent from these analyses that the sap contains less inorganic substances and more organic materials in the earlier part of the season than at the later dates. This is explained by the facts that organic materials accumulate in the woody tissue of perennial plants during the summer, and that they are rapidly removed to the growing regions with the opening of the following spring; it is at the expense of this accumulated food that spring leaves are formed. After the leaves develop, the sap contains inorganic substances mainly, and spring bleeding thus becomes transformed into summer bleeding.

The term bleeding thus denotes the exudation of sap from the woody tissues of wounded plants, brought about by the absorption of water and dissolved mineral substances by the parenchymatous cells of the root, and the movement of this solution into the vessels of the xylem, in which it is carried upward to the wound. The causes upon which this phenomenon is dependent have not yet been found out.[q]

(*c*) *Movement of water in the stem*[1] depends upon a number of conditions. Water moves upward in the xylem, as was shown in Malpighi's girdling experiment. Sach's theory, which supposed that it traverses the vessel walls, was proved untenable and is no longer upheld. The ascending current moves in the lumina of the vesssels and tracheides, as is shown by the fact that wilting promptly occurs when these are plugged. The following experiment demonstrates this. A mixture of 20 parts of gelatine in 100 parts of water, melting at 33° and still liquid at 28°C. (at which temperatures plant tissues is not at all injured) is prepared, and enough India ink is added to make the preparation readily visible in the vessels. The stem of a leafy shoot is cut under this preparation, the latter having been warmed to 33°C. The liquid rises in the vessels and is allowed to harden by cooling. A small piece is then cut from the base of the stem, to give a fresh absorbing surface, and the cut end is placed in water. After several hours wilting occurs in the leaves, while the leaves of a similar

[1] **Votchal,** Ueber die Bewegung des Wassers in den Pflanzen. Moscow, 1897 (Russian).* **Böhm, Joseph,** Ueber die Ursache des Saftsteigens in den Pflanzen. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien. **48**^{I}: 10–24. 1863. **Hartig, R.,** Die Gasdrucktheorie und die Sachs'sche Imbibitions-Theorie. Berlin, 1883. **Strasburger, Eduard,** Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. (Histologische Beiträge, Heft 3.) Jena, 1891. **Askenasy, E.,** Ueber das Saftsteigen. Verhandl. Naturhist.-Med. Ver. Heidelberg, n. F. **5**: 325–345. [Gesammtsitzung vom 7. Dez., 1894, und 1. Febr., 1895. Heft 4, dated 1896.] **Idem,** Beiträge zur Erklärung des Saftsteigens. *Ibid*, **5**: 429–448. [Gesammtsitzung vom 6. März, 1896. Heft 4. Vol. dated Heidelberg, 1897.] **Godlewski, E.,** Zur Theorie der Wasserbewegung in den Pflanzen. Jahrb. wiss Bot. **15**; 5 69–630. 1884. [**Schwendener, S.,** Untersuchungen über das Saftsteigen. Sitzungsber. (math.-naturw. Mitth.) K. Preuss. Akad. Wiss. Berlin **1886**: 355–396. 1886. **Idem,** Vorlesungen über mechanischen Probleme der Botanik. Leipzig, 1909.]

[q] Molisch showed that the phenomenon of sap exudation from holes and cuts in the upper regions of palm stems is not due to a pressure normally present in the plant, but that the pressure here indicated is brought about as a result of wounding. The cells near the cut surface undergo an alteration, and bleeding begins only after enough time has elapsed to allow this alteration to occur. The altered cells resemble gland cells and secrete the liquid. But it appears improbable that all the cases of bleeding are to be thus explained. See: **Molisch, Hans,** Botanische Beobachtungen auf Java. (III. Abhandlung.) Die Secretion des Palmweins und ihre Ursachen. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **107**^{I}: 1247–1271. 1898. **Idem,** Ueber localen Blutungsdruck und seine Ursachen. Bot. Zeitg. **60**: 45–63. 1902.

branch, the vesesls of which are not thus plugged, may remain turgid for a number of days.[1]

Air as well as water is present in the vessels and is very much rarefied at times, as was shown by Höhnel's experiments. To show the presence of water in the vessels, a piece is removed from a young stem by means of a double pair of shears, so arranged that the two cuts are made at the same time. From the piece thus obtained, longitudinal sections are prepared and examined under the microscope, of course without any addition of water. If the two cuts are not made simultaneously no water is observed in the vessels, for, because of negative pressure in the gases of the wood, air rushes into the vessels at the cut surface as soon as the incision is made, driving the water before it into other regions of the plant. The water columns in the vessels are frequently interrupted by air bubbles and these may be demonstrated under the microscope. To accomplish this the parenchymatous tissue is carefully removed from one of the woody bundles of a young stem with but little wood (*e.g.*, Begonia or Dahlia). Thus the bundle is exposed, but is uninjured and is still in connection with the rest of the plant at both ends of the preparation. Study of such preparations shows that the vessels are nearly filled with water and contain but few air bubbles in moist, cloudy weather, but that they contain less water and consequently a greater amount of air[2] on sunny days.

All the investigations that have so far been made indicate that the water columns in the vessels are not completely broken by air bubbles. Cross-sections of the vessels show that they are not perfectly cylindrical but are more or less prismatic and many-sided and that this irregularity in form is further increased by circular, spiral and other secondary thickenings of the walls. Air bubbles tend to assume a spherical form and the irregularly shaped portions of the vessels are thus not completely filled with air, so that a continuous water column results, the air bubbles being wholly surrounded by water.[r]

[1] **Errera, Léo,** Ein Transpirationsversuch. Ber. Deutsch. Bot. Ges. 4: 16–18. 1886.

[2] **Capus, Guillaume,** Sur l'observation directe du mouvement de l'eau dans les plantes. Compt. rend. Paris 97: 1087–1089. 1883.

[r] It is doubtful whether this is true when the transpiration rate is considerable and the soil fairly dry. Wherever a gas bubble occurs in a vessel it should enlarge, under these conditions, until it fills that entire vessel segment from the cross-wall below to the one above. The gas-liquid surface tension in such a case as is postulated in the text would have to be as great as the sum of the gas pressure in the enlarged bubble and the tensile stress exerted upon the water by the transpiration process going on above. The gas pressure in the bubble must be less than a single atmosphere, but the magnitude of the tensile stress is at least more than equivalent to an atmosphere. Thus the sum just mentioned is frequently of the order of several atmospheres and is surely of greater magnitude than the gas-liquid surface tension. It follows that the bubble must enlarge until its surface film comes into contact with the surrounding vessel walls at every point; thus reinforced, the surface layer of the liquid can withstand the great attraction exerted by the stressed water-mass, and the gas bubble does not expand farther. When the water has been under stress for a sufficient time there should be no free water between cell walls and gas at any point in the entire plant body; all such surfaces should be cell-wall surfaces, at which the liquid surface is held by the force of imbibition. Indeed, this condition would probably be attained by the action of the gas pressure within the bubble, before any stress developed in the liquid at all. The picture presented in the text at this

Votchal has carried out a thorough investigation upon the transmission of pressure by wood containing both air and water. [See note 1, p. 131 for reference]. Portions about 2 m. in length, from saplings or branches, were placed in a horizontal position and water was forced through them from one end to the other, by means of water or mercury pressure applied through glass tubes suitably attached. The rate of entrance of water at one end and that of exit at the other vary in a regular manner for a time after pressure is first applied. Votchal's representation of these variations is reproduced in the diagram of Fig. 82. The variation in the entrance rate, at the end where pressure is applied, is shown by the line α. This rate first increases with remarkable rapidity and soon attains a rather high value (α), but this high rate is maintained only during several hundredths of a second after the pressure is applied. The next stage ($\alpha\beta$) shows a decreasing rate and is of longer duration, continuing for from one-half to two minutes. In the third stage ($\beta\gamma$) the velocity continues to fall, but more slowly and gradually, and it finally assumes a constant value. In short pieces of stem the final constant rate is attained after five minutes, but

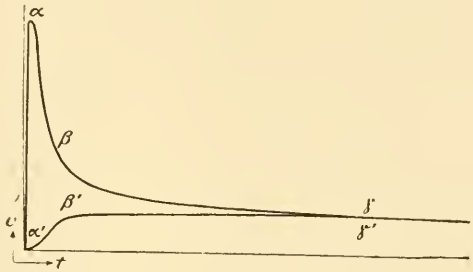

FIG. 82.—Diagram showing variations in rates of entrance and exit of water moving under pressure through a section of woody stem. (*After Votchal.*)

with longer pieces this period may be prolonged. The simultaneous variation in the rate of exit, at the opposite end of the piece of stem, is shown by the line $\alpha'\beta'$. The velocity of movement here increases very slowly, gradually attaining a value equal to that of the rate of entrance at the other end. When the two rates become equal, the two curves become coincident, and water

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point can be true only with comparatively low transpiration rates, and with comparatively ready entrance of water into the vessels below. The compound water column of the stem is not broken in all vessels at the same level, however, and the transpiration stress is transmitted laterally from the water of one vessel to that of adjoining ones, *around* the gas-filled vessel segments. These matters have been very thoroughly treated by Dixon, and Overton and Renner have each brought forward additional convincing arguments in favor of the general interpretation adopted in the present note. See: **Dixon, H. H.**, Transpiration and the ascent of sap. Prog. rei bot. **3**: 1–66. 1909. **Idem**, Transpiration and the ascent of sap in plants. London, 1914. **Renner**, 1910. [See note *k*, p 138.] **Idem**, 1911, *1*, *2*. [See note *j*, p. 137.] **Idem**, 1912, *1*, *2*. [See note *k*, p. 138.] **Idem**, Theoretisches und Experimentelles zur Kohäsions-theorie der Wasserbewegung. Jahrb. wiss. Bot. **56**: 617–667. 1915. **Holle, H.**, Untersuchungen über Welken, Vertrocknen und Wider-straffwerden. Flora **108**: 73–126. 1915. **Overton, J. B.**, Studies on the relation of the living cells to the transpiration and sap-flow in Cyperus. Bot. gaz. **51**: 28–63, 102–120. 1911.—*Ed.*

is then moving through the piece at a uniform rate throughout. Similar experiments with tubes filled with sand containing air and impregnated with water gave concordant results with those obtained with the pieces of stem. Votchal conceives that air bubbles in the wood act simply as resilient springs that transmit and distribute the thrust imparted to them more slowly and evenly than would a continuous, homogeneous water column. The effective forces applied at the ends of the conducting channels—*i.e.*, the force of foliar transpiration and that of root pressure—furnish energy to account for the ascending water current in plants. Root pressure, produced by osmotic forces, exerts a pressure upon one end of the water column in the wood, while evaporation of water from the leaves establishes traction at the opposite end.[s]

A simple experiment (Fig. 83) indicates the magnitude of the force that draws water into the leaves to replace that lost by evaporation. If the cut end of a leafy branch or stem is carefully sealed to the upper end of a glass tube filled with water, and if the lower end of the tube dips into mercury, then mercury is drawn up into the tube, replacing the water absorbed at the cut surface, which in turn replaces that lost by evaporation from the leaves. In Böhm's[1] experiments the mercury column rose 86 and even 90 cm. in the tube, thus considerably exceeding the height of mercury column supported by atmospheric pressure upon the free mercury surface below. Askenasy's[t] experiments indicate that the rise of the mercury column here shown has a simple physical cause. In these experiments the upper, broad portion of a glass funnel, the neck of which was fused to a long glass tube, was filled with a thick layer of plaster of Paris; when the plaster hardened the apparatus was filled with water, the glass tube dipping into mercury below. As water evaporated from the plaster surface the mercury rose in the tube and attained a height of 82 cm., which is, here also, noticeably greater than that attained under the action of atmospheric pressure. The funnel may be covered with animal bladder instead of being filled with plaster (Fig. 84).[u]

These experiments indicate the great magnitude of the force of cohesion existing between the molecules of water; the water column is not broken even when it is subjected to a considerable stress. These experiments also give some idea of the magnitude of the imbibition force resident in

[1] [**Bohm, J.**, Capillarität und Saftsteigen. Ber. Deutsch. Bot. Ges. 11: 203–212, 1893.]

[s] Root pressure is not to be considered as generally important in the ascent of water through plant stems. The mere existence of "negative gas pressure" in the vessels shows that the liquid above the gas bubbles is not being forced upward by a pressure applied below. Perhaps the simplest argument in favor of dismissing root pressure from consideration in the general problem of rise of sap lies in the fact that this pressure is found to be highest when water movement is slowest and lowest when movement is most rapid.—*Ed.*

[t] **Askenasy**, 1896, 1897 [See note 1, p. 143.] **Dixon**, 1914. [See note *r*, 144.].—*Ed.*

[u] But the bladder membrane has not been recorded as ever showing a rise of the mercury column above the height of the barometer. The experiment usually fails to demonstrate this important point, even with porous porcelain or plaster of Paris; the water column almost always breaks before a stress of one atmosphere is developed. In this connection see: **Ursprung, A.**, Zur Demonstration der Flüssigkeits-Kohäsion. Ber. Deutsch. Bot. Ges. **31**: 388–400. 1913. **Idem**, Ueber die Blasenbildung in Tonometern. *Ibid.* **33**: 140–153. 1915. **Idem**, Ueber die Kohäsion des Wassers im Farnannulus. *Ibid.* **33**: 153–162. 1915.—*Ed.*

cell walls of plants and also in plaster of Paris; this force is so great that when water is removed from the cell wall by evaporation more water is immediately withdrawn from the interior of the cell in spite of the osmotic force that opposes such movement. Transpiration from the leaves, the force of imbibition in the cell walls, and the cohesion of liquid water, are therefore the main causes underlying the movement of water in plant stems. The so-called root pressure, which causes bleeding in plants, may also be involved here to some extent.[v]

The amount of water passing through the plant is important in the distribution of mineral substances throughout the organism, as well as in their absorption. Schlösing's studies with tobacco plants may serve as an illustration[1]

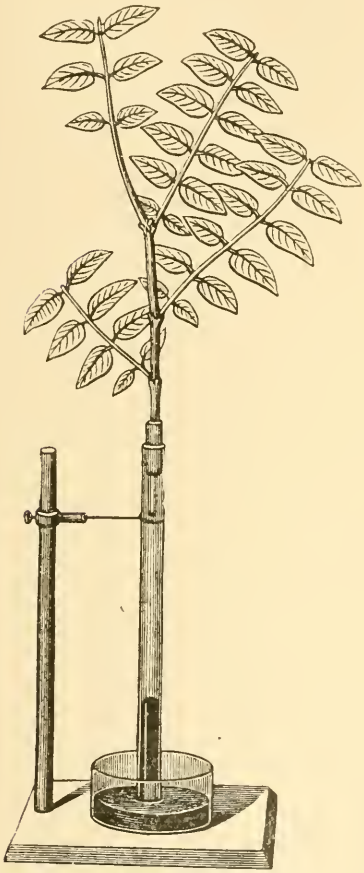


FIG. 83.—Arrangement to show rise of a mercury column caused by evaporation of water from the leaves of a cut twig.

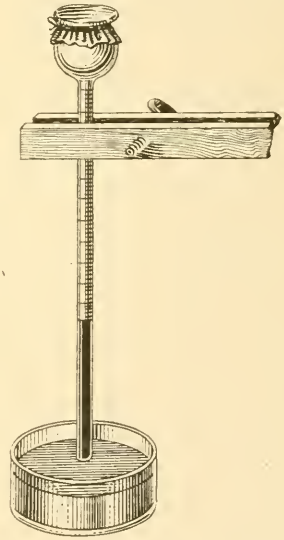


FIG. 84.—Evaporation of water through a membrane, causing rise of mercury in tube below.

[1] **Schloesing, Th.**, Végétation comparée du tabac sous glocke et à l'air libre. Compt. rend. Paris 69: 353–356. 1869.

[v] The discussion here given of the physics of the rise of the transpiration stream is fragmentary and incomplete, but it has not seemed advisable to attempt to render it much more thorough in the limited space to which editorial notes should be restricted in a translation such as the present volume. The notes that have been added to this section aim to place before the student the main points omitted by the author, and to give references to the literature, so that the best treatments of the modern phase of this much-discussed problem may be read. The writings of Dixon, Renner, and J. B. Overton, cited in note *r*, p. 144, should be referred to, at any rate. The existing text-books are all unsatisfactory in regard to this subject, the Dixon theory not yet having been adequately incorporated into any of them.—*Ed.*

of this. A portion of a plant was allowed to grow in a water-saturated atmosphere, under a bell-jar, while the remainder was exposed to natural conditions. The ash content in the leaves grown in the moist atmosphere was lower than that of the other leaves, the former being only 13 per cent., while the latter was 21.8 per cent., of the total dry weight.[w]

§5. **Movement of Organic Substances.**—Malpighi's girdling experiment, already described (page 133), indicates that organic substances move through plant stems only in the cortex. This region, however, includes many different kinds of tissue and the question arises whether the movement here considered occurs equally throughout the cortex or only through special parts of it. Hanstein[1] carried out a series of experiments in this connection and found that the removal of a ring of cortex did not always stop growth in the region below the lesion. Anatomical study of the plants that were not injured showed that some of these possessed vascular bundles in the pith as well as in the ring of vessels always found in dicotyledonous plants, while others possessed no collateral bundles and had only bicollateral ones. Girdling had no effect upon the growth of monocotyledonous plants. Hanstein concluded, therefore, that this difference between different plants, in regard to the effect of girdling, is due to the fact that all the sieve-tubes are removed in the girdling of most dicotyledonous plants, while only a part of them are removed in those dicotyledons that have vascular bundles in the pith, and in monocotyledons with bicollateral bundles. Sieve-tubes are therefore the main channels through which the movement of organic material occurs. By virtue of their anatomical structure these tubes are better suited for this movement than are any of the other tissues of the cortex. This conclusion does not at all exclude the possibility that organic substances may move by diffusion through any other living cells, especially through the very small pores by which many cell walls are perforated. A peculiarity of the movement of organic materials is that it is regulated exclusively by the activity of living cells and that it is a result of this activity. In other words, this movement is controlled by internal conditions. External conditions affect translocation only as they affect the life-processes of the cells in general. With the upward movement of the soil solution it is quite different, for, as has been seen, this is very largely dependent upon such external conditions as light, humidity, wind, etc. The movement of the soil solution has been somewhat thoroughly investigated in its general aspects, but our knowledge of the translocation of organic materials rests upon only a few well-known facts and is largely hypothetical.

The movement of organic materials has been extensively studied in connec-

[1] **Hanstein, Johannes,** Versuche über die Leitung des Saftes durch die Rinde und Folgerungen daraus. Jahrb. wiss. Bot. **2**: 392–467. 1860.

[w] But see: **Hasselbring, Heinrich,** The relation between the transpiration stream and the absorption of salts. Bot. gaz. **57**: 72–73. 1914. Hasselbring's conclusion is the direct opposite of the one reached by Schlösing. The question as to what rates of transpiration are necessary to elevate the requisite amount of salts in tall plants deserves further attention at the hands of experimenters. It appears clear enough, on *a priori* grounds, that *some* transpiration must generally give better growth than none at all, but the rates generally experienced by ordinary plants are probably much higher than the optimum.—*Ed.*

tion with seed germination. The most important work in this field was done by Sachs.[1] By means of microchemical tests applied to hand sections of seeds and seedlings, he investigated the most important organic substances (such as proteins, sugars, fats, acids, tannins), with regard to their distribution in the tissues. By comparing the distribution of these substances as shown in the seed with that exhibited in the seedling and in different regions of the older plant, Sachs reached his conclusions as to the paths of translocation. He found, for example, that the cortex contains cells that are filled with starch grains during germination, and that these cells form a continuous series (which he called the starch sheath) reaching outward from the cotyledons into all parts of the plantlet. From these observations he concluded that it is in this sheath that starch moves from the cotyledons into other regions, as growth proceeds. The sort of observations on which this conclusion was based bear, however, only upon the distribution and accumulation of the substances in question, in the various organs of the plant; the fact that a continuous series of cells all contain a certain substance does not indicate that the substance in question is moving through those cells. In the case of the starch-filled cells above mentioned, the subsequent experiments of Heine[2] showed that this material is not there in process of translocation, but that the contents of these cells represent merely local accumulations. This author removed rings of tissue from stems of young seedlings, so as to remove the starch sheath at the region of girdling, and found that such treatment neither hindered the development of the plants nor lessened the amount of starch in those regions of the sheath beyond the wound. Therefore, in this case also, the organic materials must have moved through the phloem (leptome) of the bundles, which was not injured by the girdling operation. Some of the plastic material passing through the uninjured phloem found its way to the sheath cells and there accumulated locally as starch.

There are also available some studies, by Sachs, Sapozhnikov,[3] and others, bearing upon the translocation of organic substances from the leaves, where they are formed, to other portions of the plant. As carbohydrates are produced in the leaves they continually move into the stem. Comparison of the loss of carbohydrates from attached leaves with the loss, in the same time, from similar leaves that have been detached from the plant, shows that this rate of loss is more than five times as great in the first case as it is in the second. This observation indicates clearly that translocation of carbohydrates from leaves to stem actually occurs. Carbohydrates disappear from the detached leaves only through local consumption, and the rate of its disappearance is much lower than in the case of leaves that remain attached to the plant. This movement

[1] **Sachs, J.,** Uebersicht der Ergebnisse der neueren Untersuchungen über das Chlorophyll. Flora, n. R. 20: 129–137. 1862. **Idem,** Mikrochemische Untersuchungen. *Ibid.*, n. R. 20: 289–301. 1862. **Idem.** Ueber die Stoffe, welche das Material zum Wachsthum der Zellhäute liefern. Jahrb. wiss. Bot. 3: 186–188. 1863. **Idem,** Ueber die Leitung der plastischen Stoffe durch verschiedene Gewebeformen. Flora, n. R. 21: 33–42. 1863. **Idem,** Beiträge zur Physiologie des Chlorophylls. *Ibid.*, n. R. 21: 193–204. 1863.

[2] **Heine, H.,** Die physiologische Bedeutung der sogenannten Stärkescheide. Landw. Versuchsst. 35: 161–193. 1888.

[3] **Sapozhnikov,** Die Bildung der Kohlehydrate in den Blättern and ihre Bewegung in der Pflanze, Moscow, 1890. (Russian.)* **Idem,** 1890. [See note 4, p. 31.] **Idem,** 1891. [See note 3, p. 38.] **Idem,** 1893. [See note 4, p. 31.]

of carbohydrates takes place through the phloem.[1] There is a daily periodicity in the movement of carbohydrates out of the leaf; the maximum rate of movement occurs, according to Sapozhnikov, during the early hours of the night, between 7:30 and 11:30.

In perennial plants the accumulated material formed during the summer in never wholly consumed in the same season; a large part is accumulated and remains in the plant until the following spring. The renewed activity of early spring and the development of new shoots and leaves occurs at the expense of organic material accumulated in the preceding year. Accumulation begins very early in the season in some plants—in May, for instance, in the case of the maple; in other plants it begins later—in the oak, for example, in July, and in the Scotch pine, in September. The material first accumulates in the young twigs, from which it gradually moves down the stem until the roots also are filled. Accumulation ceases at the end of the summer or in the autumn—not until the middle of October in the case of the pine, for example. In winter the accumulated material, consisting mainly of oil and starch, fills all the pith, the medullary rays, the cortex and some parts of the xylem.

The solution of the accumulated material begins in early spring. As it dissolves it passes through the medullary rays into the vessels of the xylem, in which it moves to the growing regions, as has been pointed out. If the young twigs are killed by a late spring frost, after the winter reserve has been used up, the death of the tree may follow.

Organic materials are removed from storage tissues into other tissues only when they are being consumed in the latter or are moving through these tissues into still more distant regions.[2] If the embryo is removed from a seed of maize or barley, for example, and if the remaining endosperm is planted in moist soil, then the starch of the endosperm is neither removed nor converted into sugar. If, however, the endosperm is placed on the point of a little cone of plaster of Paris, the lower end of which dips into water, the starch is then dissolved and the resulting sugar diffuses into the water below. Maize endosperm is thus completely emptied of starch in from thirteen to eighteen days and a considerable quantity of carbohydrates appears in the water. Similar experiments may be performed with bulbs, roots, rhizomes and branches. Lack of oxygen in the atmosphere about the endosperm, or the presence of ether or chloroform vapor, terminates this process.

Summary

1. **Movement of Materials in General.**—Substances enter the plant body at certain parts of its periphery, and then move to distant regions, being in many cases decomposed and their elements being recombined in various ways during their stay in the plant. Some materials remain in the plant until its death, while others are continuously or intermittently given off to the surroundings.

[1] Czapek, Friedrich, Ueber die Leitungswege der organischen Baustoffe im Pflanzenkörper. Sitzungsber. (math. naturw. Kl.) K. Akad. Wiss. Wien **106**: 117–170. 1897.

[2] Puriewitsch, K., Physiologsche Untersuchungen über die Entleerung der Reservestoffbehälter. Jahrb. wiss. Bot. **31**: 1–76. 1898.

2. **Movement of Gases.**—The internal gas spaces (the intercellular channels mentioned in Chapter V, Section 3) are continuous with the external atmosphere (through stomata and lenticels) and gas streaming as well as gas diffusion may occur through these channels. Gases enter into solution and diffuse through cell walls, protoplasm, etc., just as do other dissolved substances. Dissolved gas may go out of solution and enter the gas spaces in any region of the plant. Thus, dissolved nitrogen, oxygen, etc., may diffuse from the soil into the roots and may subsequently pass out of solution into an intercellular channel. Dissolved oxygen, produced in the chlorophyll-bearing cells of a leaf during a period of sunlight, diffuses as a solute, mainly to the periphery of a sub-stomatal gas space, where it passes out of solution and then diffuses as a gas through the stomatal pore into the surrounding atmosphere. Of course it may also diffuse in other directions through the tissues. The gas spaces of the xylem are not continuous with those of the cortex, but gases may move from one system of channels to the other, first passing into solution, then diffusing as solutes, and finally passing out of solution again. These gas spaces of the xylem are generally not intercellular; they occupy portions of the vascular channels (that is, the interiors of much elongated cells that are dead and without protoplasm and that have lost their end walls in many cases where adjacent cells were originally in contact). The pressure of the gas in the vessels is frequently much lower (especially when the transpiration rate is high) than that of the environmental atmosphere and of the intercellular cortical channels. On the other hand, the gas pressure in the xylem may sometimes be higher than that in the cortical channels (when there is sap pressure).

3. **Movement of Water and Dissolved Substances.**—Girdling experiments show (1) that water and soil solutes move from the roots to other parts of the plant body through the xylem vessels that are not blocked with gas; and (2) that organic solutes (such as sugar) move from the leaves, and from regions where such substances have been accumulated, to other regions, through the phloem of the vascular tissue.

4. **The Transpiration Stream.**—Water evaporates from the water-impregnated cell walls that bound the sub-stomatal gas spaces of leaves, and it then diffuses, as water vapor, through the stomatal openings into the external atmosphere. This process is called *stomatal* transpiration. Water also evaporates directly into the atmosphere, but at a slower rate, from the cutinized epidermal cell walls which always contain some imbibed water. This process is *cuticular* transpiration. Transpiration tends to dry the cell walls from which the water evaporates, and thus to increase the *imbibitional* attraction they exert on the liquid water within the cells and farther back in the tissues. Equilibrium tends to be reëstablished by movement of water out of the xylem vessels, through intervening cells, to the evaporating surfaces. The forces drawing water out of the vessels are very great, and they tend to *stretch* the whole *water mass* of the plant body. The vessels are sufficiently rigid to prevent their being collapsed by this inward pull on their walls, and the strain (by virtue of the cohesion of water) is transmitted to all parts, tending to remove some water from all cell walls whose outer surfaces are in contact with gas. At root surfaces this tendency results in the drawing in of water from the soil (probably carrying dissolved salts with it, in so far as the cell membranes are permeable to these solutes). As transpiration proceeds, so long as the water supply is maintained at the absorbing root surfaces (especially root-hairs), there is a flow of water into the roots, through the xylem vessels, and into the cell walls from which evaporation is occurring. This mass flow of water (carrying solutes) through the plant is called the transpiration stream. Some of the water drawn into the roots is used in tissue enlargement (which is primarily imbibi-

tional and osmotic swelling), in the photosynthesis of carbohydrates, etc., and consequently the rate of water absorption by the root system is, on the whole, for long periods, a little greater than the rate of transpiration. Also, some water is lost, in some plants, by being excreted to the exterior in the liquid form, as from hydathodes and nectaries, which excrete aqueous solution at leaf margins, on flower parts, etc. This glandular excretion of aqueous solution by hydathodes is termed *guttation*. Compared with transpiration, guttation is a slow and not very important process; it is encountered in comparatively few plants and is not maintained for long periods. Water loss through nectaries is still less significant in this connection. Sap pressure, by which the water solution of the vessels is sometimes under pressure instead of under tension (it is under pressure only when the transpiration rate is very low and the soil water supply is plentiful), appears to be due to a sort of gland action (somewhat like that of hydathodes on leaf margins) in the tissues of the roots, etc., resulting in the active forcing of solution from the cortex into the xylem vessels; the water thus forced into the xylem is derived from the surrounding tissue, and ultimately from the soil. Bleeding, as of cut grape shoots in early spring, is partly or wholly due to sap pressure. Sap pressure does not occur when the transpiration stream is rapid; at such times the solution in the xylem vessels is under tension; therefore this phenomenon cannot generally be the cause of the rise of sap in stems. This rise is directly due to the removal of water from the xylem above, to the tensile or stretching strain transmitted through the water of walls, protoplasm, vacuoles, etc., in all directions, and to the inward flow from the soil adjacent to the root surfaces. The molecular physics of sap pressure, gland secretion, etc., is not yet understood.

The rate of transpiration nearly controls the rate of water absorption in ordinary plants with a plentiful water supply. When plants are well supplied with water at the absorbing surfaces of the roots, the rate at which water is evaporated from leaves and stems is dependent on several conditions, which may be grouped as *internal* and *external*. Among the internal conditions are: The structure of the plant, the kind of epidermis, the distribution, size, and open or closed condition of stomata, the degree of water saturation of the tissues, the power of the foliage to absorb solar radiation, the rate of water movement from roots to transpiring surfaces, etc. Many (but not nearly all) stomata open and close according to conditions. Such stomata usually open when the light intensity increases about dawn, and close more or less completely with the diminution of light intensity in the evening. They also usually close when wilting approaches. Generally the guard cells are more turgid when the pores are open. Stomatal movement is due to changes in the turgor relations (tensions) between the guard cells and the other epidermal cells.

External conditions influencing the transpiration rate, when the root surfaces are well supplied with water, are the evaporating power of the air (air temperature, air humidity, air movement) and the intensity of absorbed sunlight. Over 25 per cent. of the radiant energy absorbed may be converted into the latent heat of water vapor in this way, without considerable change in the temperature of the foliage. When the supply of water to the absorbing roots is not adequate, the rate of this supply greatly influences the transpiration rate by limiting the rate of absorption by the roots. Plants usually transpire more than they absorb during the day and absorb more than they transpire during the night.

There are usually several hundred stomata per square millimeter of leaf surface, the stomata being frequently more numerous on one leaf surface than on the opposite one. For plants of the same kind, all with the same environment and all having

been grown under the same condition complex, the amount of water lost per day is about proportional to the extent of the leaf surface. According to Wollny, a maize plant transpired nearly 13 liters of water during its entire growing season. A pea plant correspondingly gave off nearly 4.5 liters. The transpiration rate is very important in determining the rate at which dissolved salts, as well as water, enter the roots from the soil, also in determining the rate at which dissolved salts are earried to the leaves. The dissolved material is left in the leaves when the water evaporates, and old leaves generally have a large salt content.

5. Movement of Organic Substances.—Organic materials (such as sugars, etc.) must be in aqueous solution to move from one region of the plant to another. Evidence points to the sieve-tubes of the phloem as the main path of movement of these solutes. They may diffuse, however, in all directions, so far as the protoplasmic membranes and cell walls are permeable to them. Also, some organic materials move in the transpiration stream, through the xylem vessels.

Carbohydrates produced by photosynthesis in the cells of green leaves, in sunlight, diffuse outward and move to other parts of the plant through the phloem. They, and other organic materials, frequently accumulate in storage tissues, often going out of solution there (*e.g.*, starch). Such accumulations usually dissolve again later, and move once more when new growth begins. The molecular physics of this movement through the phloem is not understood; the rate of the movement is too great to be accounted for by simple diffusion.

Materials enter the plant body and move about therein according to the principles and considerations that have been briefly stated. It remains to consider their *exit* from the plant body. As has been shown, water is almost continually being given off to the surrounding air by ordinary plants (transpiration, guttation). It has also been mentioned that salts, sugars, etc., are given off to a small degree through guttation and gland action. Oxygen passes from green leaves into the air during sunlight periods, and some carbon dioxide escapes in a similar way during periods of darkness. Very small amounts of volatile materials besides water, carbon dioxide, and oxygen, are given off by transpiration (volatile oils recognized as odors, etc.). Salts and organic substances are given off when flowers, fruits, leaves, bark fragments, etc., fall away. Roots regularly give off carbon dioxide, sometimes organic acids and their salts. Numerous materials are given off to the soil when roots, root hairs, and the tissues of the root-caps die and decay. The amount of nitrogenous material in the soil is often markedly increased in this manner by the growth and decay of legume roots with tubercles. Finally, the material in the plant body is ultimately released to the environment after the death of the plant, the various substances being subsequently decomposed by the action of other organisms, such as bacteria, animals, etc.

CHAPTER VII

MATERIAL TRANSFORMATIONS IN THE PLANT[1]

§1. **The Cell as the Physiological Unit.**[2]—Every plant is composed of one or more cells, each of which consists essentially of cytoplasm and nucleus. Observations and experiments have shown that the life of the cell depends upon the activities of these two parts and that the other parts of the cell are formed by these. The life of a many-celled plant is thus nothing but the sum total of the lives of its individual cells. For this reason the cell may be characterized, as the elementary organism.[3] We know of no organism with a structure simpler than that of a single cell.

The nucleus and the cytoplasm both have peculiar internal structures, and their chemical nature is very complicated and not well understood. The dried plasmodium of the slime-mould *Æthalium septicum*, consisting almost entirely of cytoplasm and nuclei, has the following chemical composition expressed, in percentage of dry weight:[4]

Proteins	40	Cholesterin	2.0
Albumins and enzymes	15	Resins	1.2
Other nitrogenous compounds	2	Calcium salts (except $CaCO_3$)	0.5
Fats	12	Other salts	6.5
Carbohydrates	12	Undetermined materials	6.5

The cytoplasm and nucleus thus consist mostly of proteins—very complicated nitrogenous compounds, many of which contain phosphorus. After treatment of proteins with gastric juice or trypsin there remains an undissolved residue containing nucleic acid. The nucleus, the cytoplasm, chloroplasts, leucoplasts, and all other living constituents of the cell are only partially dissolved in gastric juice (exceptions to this statement are very rare). On the other hand, the simple proteins (constituents of aleurone grains, albumin crystals, etc.), are completely soluble in gastric juice. The amount of simple proteins in cytoplasm and nucleus is so small that it cannot be determined at all by microchemical methods, or this is possible only with special precautions.

[1] **Euler, H.,** Grundlagen und Ergebnisse der Pflanzenchemie. 2 v. Braunschweig, 1908–1909.

[2] **Verworn, M.,** Allgemeine Physiologie. 5 Aufl. Jena, 1909. [**Idem,** General physiology. Translated by F. S. Lee, from the 2nd Ger. ed. XVI + 599 p. London, 1899.] **Reinke, J.,** Einleitung in die theoretische Biologie. 2 Aufl. 578 p. Berlin, 1911. **Hofmeister, F.,** Die chemische Organisation der Zelle. Braunschweig, 1901. [**Höber,** 1914. (See note 1, p. 119.)]

[3] **Brücke, Ernst,** Die Elementarorganismen. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien 44^{II}: 381–406. 1861.

[4] Reinke, 1881. [See note 1, p. 30.]

§2. **Proteins.**—The proteins are chemically the most complicated constituents of the plant.[1] They accumulate to the greatest extent in the protoplasm of resting cells and cells where physiological activity is just beginning. The diagrams of Fig. 85 represent stages in the development of a dicotyledonous seedling: *I* is a young embryo, *II* is a developed embryo, and *III* is a germinated seedling. The parts rich in proteins are shown in black. These parts are the youngest organs of the plant, and are either in the resting condition or are just beginning to grow. The shaded areas represent parts containing smaller

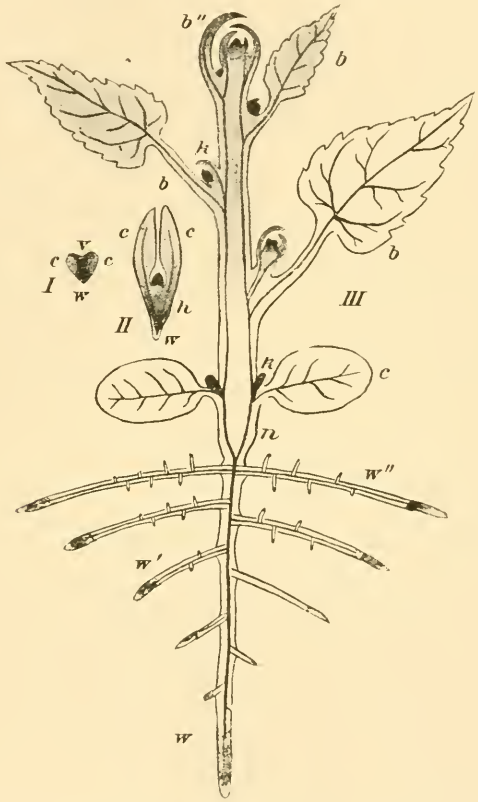


FIG. 85.—Diagrams showing stages in the development of a dicotyledonous seedling, and distribution of proteins. (*After Sachs.*)

[1] For the literature of proteins see: **Hammarsten, O.**, Lehrbuch der physiologischen Chemie. 4 te Aufl. Wiesbaden, 1899. [**Idem**, A text-book of physiological chemistry. Tr. by J. A. Mandel from 8th Ger. ed. (7th Eng. ed.) New York, 1914.] **Haliburton W. D.** A text-book of chemical physiology and pathology. 874 p. London, 1891. See p. 111–142. **Cohnheim, Otto,** Chemie der Eisweisskörper. 315 p. Braunschweig, 1900. **Griessmayer, Victor,** Die Proteide der Getreidearten. Heidelberg, 1897. [**Czapek, F.**, Biochemie der Pflanzen. 1 te Aufl. 2 v. Jena. 1905. **Idem,** same title. 2 te Aufl. Jena, 1913. (Only 1st v. (828 p.) has appeared.)] **Abderhalden, E.**, Lehrbuch der physiologischen Chemie in dreissig Vorlesungen. Berlin, 1906. **Idem,** Handbuch der biochemischen Arbeitsmethoden. 8v. Berlin, 1910–1915. [**Euler,** 1908–1909. [See note 1, p. 154.] **Hofmeister,** 1901. [See note 2, p. 154.] **Grafe,** 1914. [See note *a*, p. 83.] **Haas and Hill,** 1921. [See note 3, p. 6.] **Osborne, Thomas B.**, The vegetable proteins. London and New York, 1909. **Plimmer, R. H. Aders,** The chemical constitution of the proteins. London and New York. 1908.]

amounts of proteins and these are the regions of most active growth. The unshaded parts represent fully grown tissues, which contain only a very small amount of proteins, these substances having disappeared during the growth process. An exception to this statement are full-grown leaves, which contain much protein material in their chloroplasts. These diagrams show not only the protein contents but also the growth activities of the different parts of the plant.

The principal chemical reactions of proteins are given below.[a]

1. With copper sulphate and caustic potash solution, a dark violet color is produced (biuret reaction). Albumoses and peptones give a red color with this reagent. This reaction is of special importance, since it serves as a means of distinguishing the albumins from their cleavage products. Excess of copper sulphate is to be avoided, since the blue color of this salt may obscure the result.

2. Heating with strong nitric acid gives a deep yellow color, which changes to orange-red upon treatment with an excess of ammonia (xanthoproteic reaction).

3. Heating with Millon's reagent gives a red color (Millon's reaction).

4. With α-napthol and concentrated sulphuric acid a blue-violet color is produced (Molisch's furfurol reaction).

5. Boiling with fuming hydrochloric acid gives a bluish-violet color (Liebermann's reaction).

[a] The following additions may be useful. (1) For the biuret test, add strong KOH solution and follow with weak $CuSO_4$ solution. A partially decomposed protein—such as peptones—gives a pink or purplish-red color. Gies and Kantor give directions for a single solution suitable for this test. (**Gies, W. J.,** and **Kantor, J. L.,** Methods of applying the biuret test. Biochem. bull 1: 264–269. 1911.) To 1000 cc. of 10-per cent. aqueous solution of NaOH add 25 cc. of a 3-per cent. solution of $CuSO_4$, a few cubic centimeters at a time, with thorough shaking after each addition. Filter through glass wool if necessary. The biuret test, as well as many other microchemical reactions, may be influenced by other reactions, the possible occurrence of which must be considered. (See: **Mathewson, C. A.,** A study of some of the more important biochemical tests. Biochem. bull. 2: 181. 1912.) Biuret is represented by the formula, $NH_2CO–NH–CONH_2$. In treating sections, a solution of copper hydrate in KOH solution may be used. It is sometimes better to warm the section in weak KOH solution, wash in water and treat with $CuSO_4$ solution, after which it is again washed and then examined in KOH solution. (3) Millon's reagent is a solution of mercuric nitrate and nitrous acid. To prepare it, dissolve (in fume cupboard) mercury in twice its weight of strong HNO_3 (spec. grav. 1.42), and then dilute the solution to three times its volume, with water. This reaction and the xanthoproteic reaction are dependent on the tyrosin or tryptophan group in the protein molecule. (4) For the furfurol reaction, add a few drops of 10–20-per cent. alcoholic solution of α-naphthol, and then slowly add concentrated H_2SO_4. The color reaction appears at junction of the two liquids. If thymol is used instead of α-naphthol a carmine color is produced. (5) Liebermann's reaction is used with material that has previously been extracted with alcohol and ether, and it appears to be due to glyoxylic (glyoxalic) acid present in the ether, this acid reacting with the tryptophan group of protein. (6) For Adamkiewicz's reaction, material is extracted with ether to remove fat, dried and then extracted with glacial acetic acid. The concentrated H_2SO_4 is added slowly and the color appears at the junction of the two liquids. Here, also, the reaction seems due to glyoxylic acid (present in the acetic acid).—*Ed.*

6. Glyoxylic acid and concentrated sulphuric acid produce a beautiful bluish-violet color (Adamkiewicz and Hopkin's tryptophan reaction).

The method of Stutzer[1] may be used for the quantitative determination of proteins. This depends upon the fact that with copper hydroxide these substances form a compound that is insoluble in water. The determination is carried out as follows. The triturated plant tissue is boiled with water and the extract is then treated with copper hydroxide. The precipitate is filtered off with hot water and is then washed with alcohol and dried. This precipitate contains all the protein material. The other nitrogenous substances of plants form water-soluble compounds with copper hydroxide and are thus removed in the filtrate. For the determination of nitrogen in the precipitate the well-known method of Kjeldahl may be used. The nitrogen of most organic substances is converted into ammonia by boiling with fuming sulphuric acid and thus remains in the flask as ammonium sulphate, which may then be determined by any of the usual methods. From the result is calculated the amount of protein nitrogen originally present.

If the entire nitrogen content is determined for one portion of the material and the content in protein nitrogen is determined for another portion, the difference between these two numbers gives the amount of the non-protein nitrogen.

The following table may serve to show the relative amounts of protein nitrogen and of non-protein nitrogen contained in different plants. The quantities are given as percentages of total nitrogen present. A considerable amount of nitrogen is seen to be present in simple compounds.

	Protein Nitrogen	Non-protein Nitrogen
Vetch	67.2	32.8
Young alfalfa	73.1	26.9
Potato tubers (July 7)	58.7	41.3

From a physiological viewpoint one must distinguish between two groups of proteins: the simple proteins or albuminous bodies, and the conjugated proteins or combination of simple proteins with other substances. The simple proteins are reserve foods (as, for example, the albumin of aleurone grains), and the compound proteins are essential in the life of the cell. The latter form the principal non-aqueous component of protoplasm, as is evident from the analysis given on page 154.[b]

The simple proteins may be grouped as follows.[c]

[1] **Stutzer, A.**, Untersuchungen über die quantitative Bestimmung des Protein Stickstoffs und die Trennung der Proteinstoffe von anderen in Pflanzen vorkommenden Stickoff-Verbindungen. Jour. exp. Landw. **28**: 103–123. 1881. **Idem**, Untersuchungen über die Verdaulichkeit und die quantitative Bestimmung der Eiwissstoffe. *Ibid.* **29**: 473–492. 1881.

[b] Since our knowledge of plant proteins rests almost wholly upon the investigation of seeds, a general classification based on physiological properties is not yet possible. For a discussion of this point see: **Osborne**, 1909. [See note 1, p. 155.]—*Ed.*

[c] This discussion is mainly based on our knowledge of animal proteins; plant proteins appear to differ from these in many respects. See **Osborne**, 1909. [See note 1, p. 155]. **Haas** and **Hill**, 1921. [See note 3, p. 6.]—*Ed.*

1. *Albumins.*—The albumins are soluble in pure water and may be precipitated by saturation of the solution with ammonium sulphate. They are coagulated by boiling or by treatment with alcohol.

2. *Globulins.*— The globulins are insoluble in pure water but are soluble in solutions of neutral salts (sodium chloride, ammonium chloride, magnesium sulphate, etc.). They are completely precipitated by a half-saturated solution of ammonium sulphate and are coagulated by boiling or by addition of alcohol.

3. *Albuminates.*—These are formed when albumins and globulins are treated with weak alkalies (alkali albuminates), or with weak acids (acid albuminates, or syntonins). They are insoluble in water and in solutions of neutral salts but are soluble in weak acids and alkalies; they are not precipitated by boiling but are salted out by saturation with ammonium sulphate. They coagulate with excess of alcohol.

4. *Albumoses and Peptones.*—These compounds are the first products of the hydrolytic cleavage of proteins by enzymes. The albumoses are precipitated by ammonium sulphate, while the peptones remain in solution.

The proteins occurring in plants have been only partially worked out,[1] even for seeds. The simple ones may be illustrated by the phytoalbumins, the phytoglobulins, and the peptones.[d]

The phytoalbumins are not of common occurrence[2] and in most cases have not been identified with absolute certainty. The proteins present in the cell sap are usually globulins, since they are soluble only in the presence of salts and are obtained as a precipitate by dialysis.

The phytoglobulins are better understood.[3] They appear to compose the principal reserve proteins of some seeds. Seeds of *Lupinus luteus* may be mentioned as one of the best materials for the demonstration of phytoglobulin.

[1] **Abderhalden, Emil,** Biochemisches Handlexikon. Berlin, 1911. Vol. 4.

[2] **Martin, Sidney H. C.,** The nature of papain and its action on vegetable proteid. Jour. Physiol. 6: 336–360. 1884–1885. Green, J. R., Proteid substances in latex. Proc. Roy. Soc. London 40: 28–39. 1886. **Vines, S. H.,** and **Green, J. R.,** The reserve proteid of the asparagus root. *Ibid.* 52: 130–132. 1893.

[3] **Weyl, Th.,** Beiträge zur Kenntniss thierischer und pflanzlicher Eiweisskörper. Zeitsch. physiol. Chem. 1: 72–100. 1877–1878. **Palladin, W.,** Beiträge zur Kenntniss der pflanzlichen Eiweissstoffe. Zeitsch. Biol. 31: 191–202. 1895. **Abderhalden,** Lehrbuch, 1906. [See note 1, p. 155.] [Also see **Osborne,** 1909. [See note 1, p. 155.]

[d] The following additional information may help the reader to form a more concrete picture. (1) Albumins are plentiful in animals but generally seem to occur only in small quantities in plants. Examples of plant albumins are legumelin, from pea seeds, and leucosin, from the seeds of wheat and other grains. (2) Globulins are the main reserve proteins in seeds, excepting those of the cereals. Examples are: excelsin, from the Brazil nut (*Bertholletia excelsa*); legumin from pea seeds and seeds of other legumes; edestin, from hemp seeds (*Cannabis sativa*); conglutin, from lupine seeds. (3) Metaproteins is a better term than albuminates. (4) Proteose is the general term, so that albumose results from splitting of albumin, and globulose from splitting of globulin. It may be mentioned that the gluten of wheat, etc., is largely composed of glutelins and gliadins, two other groups that might be inserted between (2) and (3) in the text. To the latter group, besides gliadin proper (which occurs in wheat and rye), belong also hordein (in barley), and zein (in wheat and maize). It should be remembered that the classifications of proteins are based on temporary needs of description; the chemical knowledge necessary for a really satisfactory classification is still lacking.—*Ed.*

The finely powdered seeds are treated with a 10-per cent. solution of sodium or ammonium chloride and the solution is filtered off after twenty-four hours. The filtrate is then dialysed, Kühne's dialyser being well suited to this purpose (Fig. 86). The solution is placed in a tube of parchment paper surrounded by running water. The water enters through the funnel, in the diagram, and escapes through the lateral tube. After two or three days the globulin is found to have settled upon the bottom of the parchment paper tube, as a tough, viscous mass, insoluble in pure water but soluble in solutions of neutral salts. The globulins obtained from different plants are not identical. So are distinguished, for example, edestin (from fatty seeds, such as those of hemp, *Cannabis sativa*), legumin (from pea and other legume seeds), and conglutin (from lupine seeds). The phytoglobulins are not altogether the same as the typical animal globulins.

Peptones are present in plants only in very small amounts. To isolate these Neumeister[1] has made use of their solubility in saturated ammonium sulphate solution. All other proteins are precipitated by this salt. Aqueous extracts of seeds and plants are saturated with solid ammonium sulphate and then filtered. The filtrate gives the color reaction of the biuret test for peptones. The plants studied by Neumeister may be assorted into two groups according to their peptone content. Seeds of Papaver (poppy), Beta (beet), Hordeum (barley), Zea (maize), and Triticum (wheat), contain no trace of peptones; these substances being here detected only during germination. In seeds of Lupinus (lupine), Vicia (vetch), and Avena (oats), however, which belong to the second group, peptones are more plentiful than in the seedlings. The peptones thus act here as reserve food and are gradually used up during germination.

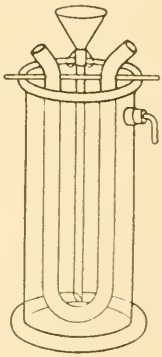


Fig. 86.—Kühne's dialyser.

An idea of the structure of the proteins may be derived from the study of their cleavage products. The products of complete hydrolysis,[2] obtained by continued boiling with concentrated acids and alkalis, or through enzymatic decomposition, are mainly amino acids. The following principal products of the hydrolysis of simple proteins have already been isolated and identified.[e]

1. Aliphatic Compounds

A. Monoamino acids

Glycocoll (α-amino-acetic acid), CH_2NH_2COOH.

d-Alanin (α-amino-propionic acid), $CH_3CHNH_2\ COOH$.

l-Serin (α-amino-β-hydroxypropionic acid, α-amino-β-lactic acid), $CH_2OHCHNH_2COOH$.

[1] **Neumeister, R.,** Ueber das Vorkommen und die Bedeutung eines eiweisslösenden Enzyms in jugendlichen Pflanzen. Zeitsch. Biol. 30: 447–463. 1894.

[2] **Abderhalden. E.,** Neuere Ergebnisse auf dem Gebiete der speziellen Eiweisschemie. Jena, 1909.

[e] The classification has been made clearer than in the German text, and the formulas are here introduced. Formulas are more clearly given in: **Mathews, Albert P.,** Physiological Chemistry. 3d ed. 1154 p. New York, 1920.—*Ed.*

d-Valin (α-amino-isovaleric acid), $\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CHCHNH_2COOH$.

l-Leucin (α-amino-isocaproic acid, α-amino-isobutyl-acetic acid),

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CHCH_2CHNH_2COOH.$$

d-Isoleucin (β-methyl-β-ethyl-α-amino-propionic acid),

$$\begin{matrix} CH_3 \\ C_2H_5 \end{matrix}\!\!>CHCHNH_2COOH.$$

l-Aspartic acid (α-amino-succinic acid), $COOHCH_2CHNH_2COOH$.

d-Glutamic acid (α-amino-glutaric acid), $COOHCH_2CH_2CHNH_2COOH$.

B. Diamino acids

Lysin (α-ϵ-diamino-caproic acid), $NH_2CH_2CH_2CH_2CH_2CHNH_2COOH$.

d-arginin (δ-guanidin-α-amino-valeric aci),d

$$HN = C\left\langle\begin{matrix} NH_2 \\ NHCH_2CH_2CH_2CHNH_2COOH. \end{matrix}\right.$$

Cystin (α-diamino-β-dithio-dilactylic acid),

$$CH_2CHNH_2COOH—S—S—CH_2CHNH_2COOH.$$

Aromatic Compounds

l-Phenyl alanin (β-phenyl-α-amino-propionic acid),

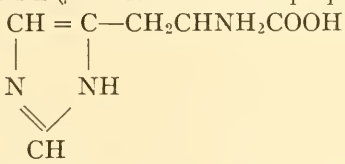
$$C_6H_5CH_2CHNH_2COOH.$$

l-Tyrosin (β-para-hydroxyphenyl-α-amino-propionic acid),

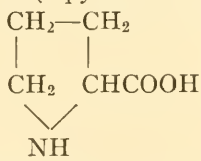
$$HOC_6H_4CH_2CHNH_2COOH.$$

3. Heterocyclic Compounds, Derivatives of Imidazol, Pyrrol and Indol

l-Histidin (β-imidazol-α-amino-propionic acid),

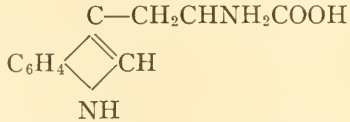


l-Prolin (α-pyrrolidin-carboxylic acid),



l-Hydroxyprolin (hydroxy-α-pyrrolidin-carboxylic acid),

l-Tryptophan (β-indol-α-amino-propionic acid).



The relative amounts of the various amino acids obtained from different proteins are not constant, as is evident from the following table, which shows these amounts for seeds of wheat and oats.

		Wheat	Oats
1. Monoamino acids	Glycin	0.90	1.0
	Alanin	4.65	2.5
	Serin	0.74	....
	Leucin	6.00	15.0
	Aspartic acid	0.90	4.0
	Glutamic acid	23.40	18.4
	Phenyl alanin	2.00	3.2
	Tyrosin	4.25	1.5
	Cystin[f]	0.02	....
	Total	42.86	45.6
2. Diamino acids	Lysin	1.90	....
	Arginin	4.70	....
	Total	6.60	....
3. Heterocylic compounds	Histidin	1.76	....
	Prolin	4.20	5.4
	Tryptophan	trace	....
	Total	5.96	5.4

The greater part of the simple proteins is thus seen to be composed of monoamino acids.

After numerous analyses had firmly established the fact that the various amino acids are to be considered as the building-stones out of which proteins are formed, Emil Fischer[1] took up the synthesis of these complicated substances from the amino acids. We now know many compounds that are produced by an amid-like linking of amino acids and Fischer has called these polypeptides. These compounds are classified according to the number of amino acids associated in their formation as dipeptides, tripeptides, tetrapeptides, pentapeptides, etc. The simplest polypeptides are crystalline compounds, but the more complicated ones, with great molecular weights, have colloidal properties, give the biuret reaction and are similar to peptones.

It is hardly to be doubted that Fischer's reasoning and methods point the way to the synthesis of proteins. The partial hydrolysis of the simple proteins has demonstrated the fact that polypeptides are undoubtedly concerned in the building up of these substances. This partial hydrolysis is effected by acids at room temperature or, at most, at temperatures not higher than 37°C. In this way polypeptides may be obtained from various simple proteins.[2] It thus appears that the simple proteins are to be considered as built up from polypeptides, which, in their turn, are products of amid-like linkings of various amino acids.

[1] **Fischer, Emil,** Untersuchungen über Aminosäuren, Polypeptide und Proteine. Berlin. 1906. **Abderhalden,** 1909. [See note 2, p. 159.]

[2] **Fischer, Emil, and Abderhalden, E.,** Bildung eines Dipeptids bei der Hydrolyse des Seidenfibroins. Ber. Deutsch. Chem. Ges. 39^{I}: 752–760. 1906. **Idem,** Bildung von Dipeptiden bei der Hydrolyse der Proteïne. *Ibid.* 39^{II}: 2315–2320. 1906. **Idem,** same title. *Ibid.* 40^{II}: 3544–3562. 1907.

[f] Cystin is a diamino acid.—*Ed.*

The simple proteins just considered act as reserve materials. The complex proteins, on the other hand, which are contained in protoplasm, sperms and egg cells, are differently constructed. Here belong nucleo-proteins, histones and protamins. Nucleo-proteins are combinations of simple proteins with other substances, they split up into simple proteins and nucleins. Nucleins are soluble in water to a considerable degree; they often fail to exhibit either the biuret or the Millon reaction. They are acid, and are not decomposed by gastric juice. Treatment with alkalies produces a splitting up of nucleins into simple proteins[1] and nucleic acids. These latter are rich in phosphorus and have very large molecular weights. The simplest formula of the nucleic acid derived from yeast cells is $C_{40}H_{59}N_{14}O_{22}\text{-}2P_2O_5$; another nucleic acid, from salmon sperm, may be expressed in simplest form as $C_{40}H_{56}N_{14}O_{16}\text{-}2P_2O_5$. The hydrolysis of nucleic acids gives phosphoric acid, pyrimidin and purin derivatives, pentoses and levulinic acid. Among these decomposition products, phosphoric acid and the purin bases—xanthin, hypoxanthin, guanin and adenin—are especially noteworthy.

For an understanding of the physiological rôle of the nucleo-proteins quantitative determinations of these compounds are requisite, but, unfortunately, no exact methods are available for the determination of nucleins. Treatment of nucleo-proteins with gastric juice leaves an insoluble residue which contains nitrogen and phosphorus. From the amount of either one of these two elements can be approximated the amount of nucleo-proteins. This method is useful only in qualitative studies, however, since different nucleo-proteins are differently affected by gastric juice. The determination of the purine bases contained in nucleo-proteins is complex and tedious. The method of Plimmer[2] has hitherto proved to be the best for this purpose. After the nucleo-proteins have been treated for from twenty-four to forty-eight hours with a 1-per cent. solution of sodium hydroxide, at 37°C., the nucleic acid remains unchanged while the entire phosphorus content of other organic compounds is split off in the form of phosphoric acid. The determination of the remaining phosphorus thus gives a starting point for the estimation of the nucleic acids. In the tips of etiolated stems of *Vicia faba*, for example, 57 per cent. of the total protein-phosphorus is present in nucleic acids and 37 per cent. is present in the indigestible protein residue.[3]

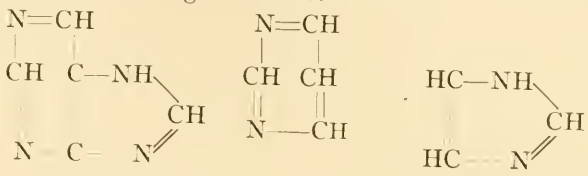
Histones and protamins have hitherto not been found in plants. These compounds can best be isolated from fish sperm. It thus seems legitimate to suppose that they may also be present in plant sperms. The decomposition products of the histones and protamins are mainly diamino acids. Among the protamins arginin is most frequently encountered (from 58 to 84 per cent.).

[1] **Altmann, Richard,** Ueber Nucleïnsäuren. Arch. Anat. Physiol. (Physiol. Abt.) **1889**: 524–536. 1889.

[2] **Plimmer, R. H. Aders,** The proteins of egg yolk. Jour. Chem. Soc. London (Transactions) 93^{II}: 1500–1506. 1908. **Plimmer, R. H. Aders,** and **Scott, F. H.,** A reaction distinguishing phosphoprotein from nucleoprotein and the distribution of phosphoproteins in tissues. *Ibid.* 93^{II}: 1699–1721. 1908. Abstracted in Biochem. Centralbl. 85^{III}: 109. 1909.

[3] **Zaleski, W.,** Ueber den Umsatz des Nucleoproteid-phosphors in den Pflanzen. Ber. Deutsch. Bot. Ges. **27**: 202–210. 1909.

It is manifest that the proteins that are most important in the life-processes are peculiarly constituted. The hydrolysis of these proteins does not primarily result in mono-amino acids, but gives, to a much greater degree, heterocyclic basic derivatives of purin, pyrimidin and imidazol. The structural formulas of these three substances are given below.



Purin Pyrimidin Imidazol

Mono-amino acids are especially scarce in fish sperms. These acids, which are so predominant in reserve proteins (following the terminology of A. Kossel[1]), are practically without importance in the formative proteins.

§3. **Enzymes.**[2]—Most biochemical reactions occurring in plants and animals can now be interpreted in terms of enzymatic activity. To be sure, success has not yet attended the effort to isolate enzymes in the free state, and the presence of an enzyme is only inferred from its specific activity. The plants in question must be killed in such a way that their enzymes are not destroyed. One of the most useful methods involves the use of a water or glycerine extract of the finely divided plant material. Brown and Morris[3] dried the plants at from 40° to 50°C. (higher temperatures are injurious to the enzymes) and showed that the powder obtained by pulverizing the dried tissues exhibited enzymatic activity. In the isolation of zymase, which accelerates alcoholic fermentation, E. Buchner found that the juice expressed from triturated yeast cells, by means of a hydraulic press, possessed the properties of the enzyme. He also employed acetone in killing the yeast cells. Palladin[4] employed a method of killing by low temperature, to demonstrate enzymes in seed-plants. The frozen plants are dead when thawed out, but the efficiency of the various enzymes has not been decreased.

As to the mechanism of their action, enzymes are to be regarded as catalyzers. Catalysis may be defined as the acceleration or retardation of an otherwise slow or limited chemical change, through the influence of a foreign substance. Many cases of the catalytic acceleration of various reactions are known in general chemistry. For example, hydrogen is but slowly formed by the action of pure sulphuric acid upon zinc. When a drop of platinic chloride solution is added, however, an energetic evolution of the gas ensues. The

[1] **Kossel, A.,** Einige Bemerkungen über die Bildung der Protamine in Tierkörper. Zeitsch. physiol. Chem. 44: 347–352. 1905.

[2] **Duclaux, E.,** Traité de microbiologie. Paris, 1898, 1899, 1900. Disastases, toxines et venins, in v. 2. **Oppenheimer, Carl,** Die Fermente. 3te Aufl. Leipzig, 1909. **Abderhalden's** Handb. 1910. [See note 1, p. 155. **Green, J. Reynolds,** The soluble ferments and fermentation. Cambridge, 1899. 2nd ed. Cambridge, 1901. [**Euler,** 1908. (See note 1, p. 154.) **Idem,** Allgemeine Chemie der Enzyme. Wiesbaden, 1910. **Idem,** General chemistry of the enzymes. Tr. from revised and enlarged Ger. ed. by Thomas H. Pope. ix + 323 p. New York, 1912.

[3] **Brown and Morris,** 1893. [See note 1, p. 28.]

[4] **Palladin, W.,** Die Arbeit der Atmungsenzyme der Pflanzen unter verschiedenen Verhältnissen. Zeitsch. physiol. Chem. 47: 407–451. 1906.

velocity of the decomposition of hydrogen peroxide by alkalies is distinctly increased by a very small amount of platinum or other metals. In both cases platinum plays the part of the inorganic catalyzer.[1] The catalytic activity, of both organic and inorganic catalyzers, depends upon the amount of catalyzer present, upon the temperature and upon the properties of the surrounding medium. Chemical reactions can not only be accelerated by foreign substances, but they can also be retarded. As an instance of this, the catalytic effect of platinum upon the decomposition of hydrogen peroxide by alkalies is greatly reduced by the presence of a trace of hydrocyanic acid, arsenic acid, hydrogen sulphide or other poisons.

Diastase is the most widely distributed of the plant enzymes. It causes the transformation of starch into glucose. A very slight amount of the enzyme is able to hydrolyze large amounts of starch; one part by weight of the powder called diastase decomposed 2000 parts of starch.

Diastase, according to investigations carried out by Baranetskii,[2] is very widely distributed in plants. It is found in especially large amounts during the germination of starchy seeds. The approximate isolation of diastase is best effected from barley malt. The malt is first digested with water, the extract is then filtered, and the enzyme is finally precipitated in the filtrate by the addition of alcohol. The white precipitate obtained in this way is purified by being repeatedly dissolved in water and reprecipitated with alcohol. The precipitate from alcohol is soluble in water, and when thus dissolved, possesses the ability to hydrolyze starch. The chemical composition of diastase appears to be very similar to that of the proteins.

The first stage of starch hydrolysis is indicated by the fact that addition of iodine to the mixture fails to give the usual blue color of starch with this reagent. In a somewhat early stage of the process, the color produced by addition of iodine is violet, but at a later stage of the hydrolysis a brown color is produced. Finally, there is no color change at all with addition of iodine. The reaction is hastened by higher temperature, up to 45° or 50°C. The decomposition of undissolved starch (starch grains) occurs only in the presence of acids (hydrochloric, formic, acetic, and citric acid), formic acid being especially active, according to Baranetskii's results. The action of diastase on starch grains *in vitro*, shows the same peculiarities as appear when the starch grains are dissolved in the seed during germination. The diastase attacks only portions of each grain first; these portions becoming transparent, glassy and giving no color with iodine; the whole starch grain becomes transparent at length, showing only a sort of framework, and finally even this is dissolved. Much information is now available upon the formation and distribution of diastase in germinating barley. The following table shows the relative amounts and distribution of diastase in Hordeum (barley) seedlings four days old.[3]

[1] **Bredig, Georg,** Anorganische Fermente. Leipzig, 1901. **Idem,** 1902. [See note 1, p. xxx.]

[2] **Baranetzky, J.,** Die Stärkeumbildenden Fermente in den Pflanzen. Leipzig, 1878.

[3] **Moritz., E. R.,** and **Morris, G. H.,** Handbuch der Brauwissenschaft (German transl. by Windisch). P. 142. Berlin, 1893.* [**Idem,** Textbook of the science of brewing. London, 1891.]

In the half of the endosperm nearest to the embryo	9.7970
In the half of the endosperm farthest from the embryo	3.5310
In the roots	0.0681
In the leaves	0.0456
In the scutellum	0.5469
Total	13.9886

It thus appears that, in such seedlings, diastase is most plentiful in the endosperm.

Diastase is less easily demonstrated in leaves of mature plants than in germinating seeds. Extracts of fresh leaves contain almost no diastase, since the enzyme diffuses hardly at all through cell walls. Brown and Morris[1] recommended the following method for obtaining it from leaves. The leaves are dried at 40° to 50°C., after which they are ground to a fine powder, which is allowed to act upon starch in water. Different leaves have different diastatic powers, as may be seen from the table given below, in which the relative efficiencies of leaf powders from five different plants are presented.

Pisum sativum (pea)	240.30
Lathyrus odoratus	100.37
Helianthus annuus (sunflower)	3.97
Syringa vulgaris (lilac)	2.52
Hydrocharis morsus-ranæ	0.26

The greater is the tannin content of leaves, the weaker is the diastatic power. Detailed researches have shown that diastase consists of a mixture of at least two different enzymes, amylase and maltase. Amylase effects the transformation of starch to maltose, which in turn is transformed into glucose through the action of maltase.

Starch is replaced by inulin in the tubers of some plants, such as Inula, Helianthus, Dahlia. The cleavage of inulin takes place through the agency of a specific enzyme, inulase. For the isolation of inulase a glycerine extract is prepared from dried sprouting tubers and the extract is dialyzed. The solution of inulase thus obtained produces hydrolytic cleavage of inulin.

Saccharase (invertase) hydrolyzes saccharose and is especially abundant in yeast. It is concentrated in the following way. Yeast that has been dried at 40°C. is heated for six hours at 100° and is then placed in water and left undisturbed for twelve hours at 40°. The preparation is then filtered and alcohol is added to the filtrate. A precipitate is thus formed, which is purified by being repeatedly dissolved in water and reprecipitated with alcohol. One part of the dry precipitate is capable of inverting 700 parts of saccharose when in solution.

Emulsin is found in sweet almonds. It splits the glucoside amygdalin into glucose, hydrocyanic acid and benzaldehyde.[g]

[1] **Brown and Morris**, 1893. [See note, 1, p. 28.]

[g] The complete hydrolysis is represented by the equation:

$$\underset{\text{Amygdalin}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Hydrocyanic acid}}{HCN} + \underset{\text{Benzaldehyde}}{C_6H_5CHO}.$$ —*Ed.*

Myrosin occurs in the seeds of black mustard. It decomposes sinigrin into mustard oil (allyl isothiocyanate), glucose and monopotassium sulphate.[h]

The decomposition of simple proteins is brought about through the agency of proteolytic enzymes. Glycerine extraction of this type of enzymes is not always successful and the method of Neumeister[1] is to be recommended here. Fresh fibrin (from blood), which has the power to absorb proteolytic enzymes from their solution, is placed in an aqueous extract of the plant material to be studied. After two hours the fibrin is removed, washed with water, and left in a weak solution of oxalic acid, in a warm place. If proteolytic enzymes were present in the original extract, the fibrin is completely dissolved after five or six hours. In the control preparation the fibrin remains practically unchanged after two days in the weak oxalic acid solution.

No proteolytic enzymes are present in resting seeds, but Butkevich[2] has isolated this kind of enzyme from germinating seeds. The sprouted seeds are dried at a temperature of from 35 to 40°C., and then pulverized, after which the mass is extracted with ether and placed in water with an antiseptic (thymol). The preparation is allowed to remain in a thermostat, with a temperature of from 35° to 40°. for several days. Auto-digestion results, accompanied by a decrease in the amount of protein material present. The proteolytic enzyme is extracted with glycerine and the extract effects a cleavage of proteins, with the formation of tryosin and leucin. Butkevich was unable to isolate asparagin, but this is readily understood, since it is not a primary product in the hydrolysis of proteins (see pp. 159–161).

Saponification of fats and oils occurs in plants through the agency of specific enzymes, the so-called lipases.[3] Lipase is now obtained for technical purposes from fatty seeds.[4]

The enzymes thus far mentioned cause various hydrolytic decompositions, but oxidizing enzymes also occur, in plants as well as in animals. Laccase was the first of these oxidases to be discovered. It causes the formation of laccol in the latex of various species of Rhus. The latex, which is originally white, changes very quickly in the presence of air and becomes black. Laccase is soluble in water and may be precipitated with alcohol. Its oxidizing effect disappears after heating to 100°C. Laccase oxidizes various aromatic compounds by means of molecular oxygen. The presence of this enzyme is shown by a blue color-reaction with a solution of gum guaiac in 60 to 80 per cent. alcohol.

[1] **Neumeister,** 1894. [See note 1, p. 159.]

[2] **Butkewitsch, Wl.,** Ueber das Vorkommen eines proteolytischen Enzymes in gekeimten Samen und über seine Wirkung. Zeitsch. physiol. Chem. 32: 1–53. 1901.

[3] **Nicloux, Maurice,** Contribution à l'étude de la saponification des corps gras. Paris, 1906.

[4] **Hoyer, E.,** Ueber fermentative Fettspaltung (2te Mittheilung.) Ber. Deutsch. Chem. Ges. 37^{II}: 1436–1447. 1904. **Idem,** same title. Zeitsch. physiol. Chem. 50: 414–435. 1906–1907.

[h] Sinigrin is potassium myronate, a glucoside, myronic acid being $C_{10}H_{17}O_9NS_2$. The hydrolysis is represented by the equation:

Potassium myronate		Glucose	Monopotassium sulphate		Allyl isothiocyanate
$C_{10}H_{16}KO_9NS_2$	$+ H_2O =$	$C_6H_{12}O_6$	$+ KHSO_4$	$+ CH_2 =$	$CHCH_2NCS$.—*Ed.*

According to the theory of Bach and Chodat[1] the oxidases are not to be considered as simple substances; they consist of peroxidases (oxidizing enzymes) and oxygenases (organic peroxides). Frequently the blue color with tincture of gum guaiac appears only after addition of hydrogen peroxide, in which cases it is evident that only peroxydase is present; hydrogen peroxide here takes the place of oxygenases, which are absent.

E. Buchner and his co-workers[2] have isolated an enzyme from yeast, which splits glucose into ethyl alcohol and carbon dioxide, the so-called *zymase*. If compressed yeast is triturated in water with quartz sand and infusorial earth (kieselguhr), and is then subjected to high pressure with a hydraulic press, a liquid is obtained that is free from cells, and that produces a very active alcoholic fermentation. For example, from 26 g. of glucose were obtained 12.4 g. of alcohol and 12.2 g. of carbon dioxide. Thus, as the theory demands, almost equal amounts of alcohol and of carbon dioxide are produced; 160 g. $C_6H_{12}O_6$ = 92 g. C_2H_6O + 88 g. CO_2.

Zymase has also been isolated[3] by treating yeast with acetone. The yeast is first pressed to remove most of the water and is then placed in a sieve and plunged in a flat dish of acetone for ten minutes. The material is then pressed again, treated with acetone and washed with ether, after which it is pulverized and dried (beginning at room temperature and ending at 65°C.). This preparation, which possesses keeping-qualities, is on the market under the name of *zymin*.[4] In sugar solutions it produces alcoholic fermentation. Lebedev[5] gives a good method for obtaining a very active enzyme from thoroughly macerated dry yeast.[6]

More extended researches[i] upon alcoholic fermentation have shown that zymase, like diastase, is not a single enzyme.[7] It is supposed that glucose is split up by dextrase into two molecules of dihydroxyacetone, $CH_2OH—CO—$

[1] **Bach, A.,** and **Chodat, R.,** Zerlegung der sogennanten Oxydasen in Oxygenasen und Peroxygenasen Ber. Deutsch. Chem. Ges. **36**: 606–609. 1904. **Chodat R.,** and **Bach, A.,** Recherches sur les ferments oxydants. Arch. sci. phys. et nat. *IV*, **17**: 477–510. 1904. **Idem,** Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. VII. Einiges über die chemische Natur der oxydasen. Ber. Deutsch. Chem. Ges. 37^{I}: 36–43. 1904. **Idem,** same title. VIII. Ueber die Wirkungsweise der Peroxydase. *Ibid.* 37^{II}: 1342–1348. 1904. **Engler, C.,** and **Weissberg, J.,** Kritische Studien über die Vorgänge der Autoxydation. 204 p. Braunschweig, 1904. **Haar, A. W. van der,** Untersuchungen über Pflanzen-Peroxydasen. I. Eine neue Methode der Peroxydasen-Gewinnung. *Ibid.* 43^{II}: 1321–1327. 1910. **Idem,** same title. II. Die Hedera-Peroxydase, ein Glucoproteid. *Ibid.* 43^{II}: 1327–1329. 1910. **Bach, A.,** Die langsame Verbrennung und die Oxydationsfermente. Fortschr. naturw. Forsch. **1**: 85–140, 1910. **Palladine, W.,** und **Iraklionoff, P.,** La peroxydase et les pigments respiratoires chez les plantes. Rev. gén. bot. **23**: 225–247. 1911. [For more recent literature see **Atkins,** 1916. [See note *o*, p. 115.]

[2] **Buchner, Eduard, Buchner, Hans,** and **Hahn, Martin,** Die Zymasegärung, Untersuchungen über den Inhalt der Hefezellen und die biologische Seite des Gärungsproblems. Munchen and Berlin, 1903.

[3] **Albert, R., Buchner, E.,** and **Rapp, R.,** Herstellung von Dauerhefe mittels Aceton. Ber. Deutsch. Chem. Ges. 35^{II}: 2376–2382. 1902.

[5] It may be obtained from A. Schroder, München, Landwehrstrasse 45.

[4] **Lebedew, A.,** Darstellung des aktiven Hefensaftes durch Maceration. Zeitsch. physiol. Chem. **73**: 447–452. 1911.

[6] This is also to be obtained from Schroder, as "Trocken-Hefe nach A. Lebedew."

[7] **Jensen, P. Boysen,** Die Zersetzung des Zuckers während des Respirationsprozesses. Ber. Deutsch. Bot. Ges. **26a**: 666–667. 1908. **Idem,** Sokkersonderdelingen under respirationsprocessen hos höjere planter. Kjöbenhavn. 1910.* **Buchner, Edward,** and **Meisenheimer, Jakob,** Die chemischen Vorgänge bei der alkoholischen Gärung. (IV. Mitteilung.) Ber. Deutsch. Chem. Ges. 43^{II}: 1773–1795. 1910.

[i] This paragraph is omitted in the 7th Russian edition.—*Ed.*

CH_2OH; dihydroxyacetone is then changed by dihydroxyacetonase into alcohol and carbon dioxide. The process of alcoholic fermentation may therefore be represented in the following way:

$$C_6H_{12}O_6 = 2C_3H_6O_3 = 2C_2H_5OH + 2CO_2$$

The parts of the plant that are rich in protoplasm usually contain appreciable amounts of *catalase*, which splits hydrogen peroxide into molecular oxygen and water. The physiological rôle of catalase is not well understood at present; it is probably connected with anaerobic processes, to which the common reduction processes[1] seem also to be related. Whether the latter are caused by specific enzymes (reductase, hydrogenase) is still undetermined. The process of reduction may be demonstrated if plant tissues are placed in a solution of methylene blue or sodium selenite, in the absence of oxygen. Methylene blue is thus bleached, while sodium selenite is decomposed with the formation of red metallic selenium. While oxidase is to be conceived as a system consisting of peroxidase with a peroxide-former (oxygenase), Bach[2] considers reductase as a combination of an enzyme with a water-splitting substance.[i]

Only the most important of the enzymes thus far discovered have been described in the preceding paragraphs, but it is probable that the living protoplasm produces specific enzymes for most of the biochemical reactions. The same organism may produce different enzymes according to the chemical nature of the nutritive material at its disposal. Thus, *Penicillium glaucum* produces saccharase when grown in a medium containing calcium lactate, casease when cultivated in milk, and lipase when supplied with monobutyrin.

Synthetic processes, as well as those of decomposition, can be brought about by enzymes. Hill,[3] for example, has found that the inversion of maltose by maltase is not complete, but proceeds to a definite equilibrium point as the velocity of the process is reduced by the accumulation of glucose. From this it is at least apparent that this is a reversible reaction. Hill proved that a concentrated glucose solution is actually transformed, in the presence of maltase, into a maltose solution. It seems plausible to suppose, in the light of the studies on this subject so far available, that enzymatic processes in general may be thus reversible.[4]

It is now possible to produce death in plants without destroying the enzymes of their tissues. Plants that have been so treated are not the same as those that have been killed in such a way as to render their enzymes inactive. (Enzymes

[1] **Ehrlich, Paul,** Das Sauerstoff-Bedürfniss des Organismus. 167 p. Berlin, 1885. **Palladin, W.,** Beteiligung der Reduktase im Prozesse der Alkoholgärung. Zeitsch. physiol. Chem. **56**: 81–88. 1908. **Zaleski, W.,** Ueber die Rolle der Reduktionsprozesse bei der Atmung der Pflanzen. (Vorläufige Mitteilung.) Ber. Deutsch. Bot. Ges. **28**: 319–329. 1910. [Appleman, Charles O., Relation of oxidases and catalase to respiration in plants. Amer. jour. bot. **5**: 223–233. 1916. (Other references are there given.)]

[2] **Bach, A.,** Zur Kenntnis der Reduktionsfermente. I. Mitteilung. Ueber das Schardinger-Enzym (Perhydridase). Biochem. Zeitsch. **31**: 443–449. 1911.

[3] **Hill, Arthur Croft,** Reversible zymohydrolysis. Jour. Chem. Soc. London **73**: 634–658. 1898.

[4] **Dietz, Wilhelm,** Ueber eine umkehrbare Fermentreaktion im heterogenen System. Esterbildung und Esterverseifung. Zeitsch. physiol. Chem. **52**: 279–325. 1907. **Loeb, Jacques,** The dynamics of living matter. 233 p. New York, 1906.

[i] The considerations of this paragraph receive more detailed attention in the following chapter.—*Ed.*

lose their power with temperatures about 100°C.) Trommsdorff[1] called the former "*abgetötet*" and the latter "*abgestorben.*"[k] If plants are killed in the proper way, the enzymes of their tissues still exhibit their characteristic properties, in the presence of air, water, and substances that are poisonous to bacteria but not injurious to the enzymes. It may seem, at first thought, that such plants should continue to carry out their general life-processes in the same manner as do living ones, so that the latter might be hard to distinguish from the former. Deeper study, however, reveals important differences. When plants are killed without the destruction of their enzymes the physiological system of the cells appear to become completely disarranged, with the destruction of the interrelations that obtain between the different constituents of the living cell. In the living organism the different cells and cell components appear to be bound together and interrelated so as to form a harmonious whole—somewhat as our solar system is unified—but the component units of a dead cell, even though its enzymes still retain their proper powers, appears to be a mass of unrelated components enclosed within a common membrane; a tissue composed of such cells is without the interrelations that make it a living tissue. Just as an atom of radium breaks down into its component particles, so does the living cell break down at death, it being the largest physiological unit of the organism. The following important differences may be noted between the enzymatic processes of dead cells and those of living ones.[2]

1. There is no correlation in activity between the different enzymes in dead cells. In living cells an enzyme remains active only so long as the products of its activity are used. In dead cells the activity of an enzyme is not regulated by that of the other enzymes. Enzyme activity is apt to be prolonged even after its products have ceased to be removed.

2. In dead cells enzymes are decomposed by other enzymes. This was clearly demonstrated by the experiments of Petrushevskaia.[3] As is well known, the respiratory activity of living yeast is increased by a rise in temperature. On the other hand, the enzymatic activity of zymin (yeast killed with acetone) is retarded by increased temperature. So, for example, 10 g. of zymin produced 706.5 mg. of CO_2 at from 22 to 23°C., while only 285.3 mg. were formed at from 33 to 34°C. This difference, amounting to 59.7 per cent., may be explained by supposing that the velocity of protein decomposition in the acetone preparation increases with higher temperature. According to Petrushevskaia only 35.9 per cent. of the protein nitrogen of the zymin was decomposed in three days at from 15 to 16°C., while 81.5 per cent. was broken down in the same time at 32°. The proteolytic enzyme appears to decompose the zymase, which is of protein nature.

[1] **Trommsdorff, Richard,** Ueber die Beziehungen der Gram'schen Färbung zu chemischen Vorgängen in der abgetöteten Hefezelle. Centralbl. Bakt. *II*, **8**: 82–87. 1902.

[2] **Palladin, W.,** Die Eigentümlichkeiten der Fermentarbeit in lebenden und abgetöteten Pflanzen. Fortschr. naturw. Forsch. **1**: 253–268. 1910.

[3] **Petruschewsky, Anna,** Einfluss der Temperatur auf die Arbeit des proteolytischen Ferments und der Zymase in abgetöteten Hefezellen. Zeitsch. physiol. Chem. **50**: 251–262. 1906–1907.

[k] While there is some usage of *killed* and *dead*, as corresponding to these German words, such a usage seems undesirable and is here avoided.—*Ed.*

3. Enzymes in dead cells are destroyed by various poisons and bacteria, that have no effect upon them in the living cell. Korsakova[1] showed that living yeast cells exhibit alcoholic fermentation in the presence of considerable amounts of sodium selenite, but the production of carbon dioxide in yeast killed with acetone is stopped at once by a trace of this substance.

The experiments described above show that life-processes are not to be interpreted simply as enzymatic activity. Enzymatic activities are regulated in living cells, and the apparently unregulated processes carried out by the enzymes of dead cells indicate that enzymes really play a subordinate rôle in the life of the organism.

Living protoplasm is not to be considered merely as a complex of heterogeneous enzymes. Enzymes are, in a manner of speaking, workers in the service of the protoplasm; they are formed by the protoplasm, used in the work that is in hand, and then imprisoned or destroyed as soon as their activities are no longer required.[l] Enzymes that have become unnecessary are rendered inactive by specific anti-enzymes; they are imprisoned, as it were, and when they once more become necessary they are rendered active again, from the condition of proenzymes, by activators or kinases. Activators or kinases on the one hand, and anti-enzymes on the other, are thus the agents through which the regulating power exerted by the protoplasm is effected.

In the animal organism, moreover, special substances are found that not only modify the activities of the various enzymes but also regulate the processes of the whole body, and even initiate the development of new organs. These substances arise in some particular organ and then migrate into far-distant regions, where they set up whole series of definite chemical reactions. Such chemical messengers have been called *hormones* by Starling.[2]

§4. Protein Decomposition in Plants.[m]—As has been stated above, proteins do not remain unchanged in plants, but are continually being broken down and again reformed.[3] Some life-processes depend upon protein decomposition and others upon protein synthesis. Etiolated seedlings and actively growing plant organs are very satisfactory subjects for the study of protein decomposition. We owe our first information regarding this decomposition to Theodor Hartig.[4] This author found an important nitrogenous substance in seedlings, which he designated by the name "Gleis." It developed later that Hartig's "Gleis" is identical with asparagin. Boussingault[5] asserted that asparagin appears in all

[1] **Korsakoff, Marie,** Ueber die Wirkung des Natriumselenits auf die Ausscheidung der Kohlensäure lebender und abgetöteter Hefe. Ber. Deutsch. Bot. Ges. 28: 334–338. 1910.

[2] **Bayliss, W. M.** and **Starling, E. H.,** Die chemische Koordination der Funktionen des Körpers. Ergeb. Physiol. 5: 664–697. 1906.

[3] **Lusk, Graham,** The elements of the science of nutrition. 2nd ed. 402 p. London and Philadelphia, 1909.

[4] **Hartig, Theodor,** Entwickelungsgeschichte des Pflanzenkeims, dessen Stoffbildung und Stoffwandlung während der Vorgänge des Reifens und des Keimens. Leipzig, 1858.

[5] **Boussingault,** 1860–1861. [See note 5, p. 2.] Vol. 4, p. 265.

[l] Of course this is a figurative way of describing these phenomena. The "necessity" for an enzyme, or the need of the work it can do is not a reason for its being produced.—*Ed.*

[m] This section is numbered §6 in the German. The numbering of the 7th Russian edition is here followed.—*Ed.*

plants that are subjected to illumination. Respiration in plants is connected with protein decomposition, and asparagin is formed as one of the main nitrogenous products. This process is to be considered as strictly analogous to the formation of urea in animals, but urea is eliminated from the animal body while asparagin is again utilized in the plant body, by means of the energy of sunlight. Pfeffer[1] has demonstrated by microchemical observation, that asparagin disappears as carbohydrates accumulate during the process of photosynthesis in sunlight, being used in protein synthesis. When seeds germinate in the dark, however, protein decomposition predominates, and asparagin therefore accumulates. Under usual conditions the synthesis and decomposition of proteins occur simultaneously, but it should be mentioned that the influence of light becomes apparent only in the later stages of germination. In the earlier stages asparagin accumulates, in light as well as in darkness. Afterwards asparagin increases in amount only in darkened plants, while lighted plants gradually lose all the asparagin that has previously been formed. These relations were long ago pointed out by Boussingault and were later verified by Meunier.[2] The following table shows some of Meunier's results with *phaseolus coccineus*. The numbers denote the relative amounts of asparagin found in plants of three different ages, in darkness and in light.

Age of Plants	Relative Amounts of Asparagin in Plants Grown in	
	Darkness	Light
days		
13	1.13	1.18
18	2.28	2.25
38	5.18	1.41

Of the seedlings eighteen days old, those in light contained as much asparagin as those in darkness. In the oldest seedlings, however, the asparagin content had markedly increased in the darkened plants but had decreased in the illuminated ones.

Pfeffer has confined his researches upon asparagin exclusively to the legumes, which are rich in this substance, but Borodin[3] showed that asparagin is very widely distributed and is probably present in the majority of plants. Under the usual conditions of plant life the detection of asparagin is frequently either very difficult or even impossible, but if the plants to be studied are placed in water culture in darkness for several days, then the carbohydrates necessary for protein formation become entirely used up and asparagin accumulates, as Borodin was able to show by microchemical tests. Along with asparagin, Borodin also found tyrosin and leucin.

Borodin's conclusions were afterwards quantitatively substantiated by

[1] **Pfeffer, W.,** Untersuchungen über die Proteïnkörper und die Bedeutung des Asparagins beim Keimen der Samen. Jahrb. wiss. Bot. **8**: 429–574. 1872.

[2] **Meunier, Fernand.,** Étude sur l'asparagine. Ann. agron. **6**: 275–281. 1880.

[3] **Borodin, J.,** Ueber ide physiologische Rolle und die Verbreitung des Asparagins im Pflanzenreiche Bot. Zeitg. **36**: 801–832. 1878.

Ernst Schulze.[1] The following experiment with oat seedlings may serve as an illustration of his work. The seedlings were first grown in light, then some of them were used for analysis while the rest were placed in darkness. After a week these also were analyzed. The numbers given below show the relative

	Original Plants	After a Week in Darkness
Total nitrogen	4.12	4.50
Nitrogen of proteins	3.51	1.46
Non-protein nitrogen	0.61	3.04

amounts of protein and non-protein nitrogen found in each of the two lots of seedlings. During the course of seven days in darkness more than half the total amount of protein material is thus seen to have been broken down.

The chemical nature of the protein decomposition products is dependent upon various conditions; with different environmental conditions very different decomposition products are produced. Oxygen is very important for the progress of protein decomposition, but Palladin[2] has shown that this process goes on also in the absence of oxygen. The following table shows the relative rates at which protein decomposition occurred in wheat seedlings grown with and without oxygen. The numbers denote percentages of total original protein decomposed during the corresponding time periods.

	Percentage of Original Protein Decomposed	
Time Period	Without Oxygen	With Oxygen
22 hours	1.1	....
1 day	3.9	7.9
2 days	15.4	17.2
3 days	26.1	....
7 days	....	54.3

The quantitative relations of the individual decomposition products are not the same in the absence of oxygen as in its presence. In the latter case asparagin is the main product while tyrosin and leucin are formed only in very small quantities. In the absence of oxygen, however, tyrosin and leucin accumulate to a marked degree while the amount of asparagin formed is quite negligible. This fact shows that the primary products of protein hydrolysis are formed only in the absence of oxygen. As long as asparagin was considered as one of these primary products it was impossible to understand how protein hydrolysis within the plant body results in the formation of asparagin, while the hydrolysis of plant proteins with acids produces but a negligible amount of aspartic acid (see page 161). The experiments described above explain this; it has been shown that asparagin arises during synthetic processes. Borodin[3] had already

[1] **Schulze, E., Steiger E., and Bossard, E.** Untersuchungen über die stickstoffhaltigen Bestandtheile einiger Rauhfutterstoffe. Landw. Versuchsst. **33**: 89–123. 1887. [**Schulze, E.,** Ueber die Methoden, welche zur quantitative Bestimmung der stickstoffhaltigen Pflanzenbestandtheile verwendbar sind. *Ibid.* **33**: 124–145. 1887.]

[2] **Palladin, W.,** Ueber Eiweisszersetzung in den Pflanzen bei Abwesenheit von freiem Sauerstoff. Ber. Deutsch. Bot. Ges. **6**: 205–212. 1888. **Idem,** Ueber Zersetzungsproducte der Eiweissstoffe in den Pflanzen bei Abwesenheit von freiem Sauerstoff. *Ibid.* **6**: 296–304. 1888.

[3] **Borodin, I. P.,** On the conditions for the accumulation of leucin in plants. [Russian.] Trav. Soc. Imp. Nat. St.-Pétersbourg **16** (Protocole): 69–73. 1885.

observed that no asparagin is formed in the absence of oxygen. The researches of Palladin have recently been repeated and substantiated by Godlewski,[1] and Butkevich[2] also obtained similar results. *Aspergillus niger* decomposes peptones to ammonia in the presence of oxygen, but only to amino acids in the absence of this element. It still remains uncertain in what way asparagin is formed from the primary products of protein cleavage, but it seems possible that here, also, an enzymatic process is involved.

The formation of the various nitrogenous cleavage products of proteins is also dependent upon the chemical nature of the nutrient medium in which the organism is grown. Butkevich[3] showed that different moulds do not produce the same cleavage products when grown in peptone solution. *Aspergillus niger* produces ammonia mainly, while *Penicillium glaucum* forms tyrosin and leucin for the most part. This difference is correlated with the acid or alkaline reaction of the substratum. Aspergillus forms a considerable amount of oxalic acid and this renders the nutrient solution acid. Penicillium produces no oxalic acid and the solution in which it is growing soon becomes alkaline, as a result of ammonia formation. If, however, Aspergillus is cultivated with an excess of calcium carbonate in the medium, then it forms considerable amounts of tyrosin and leucin, while Penicillium produces ammonia in considerable amount when the nutrient solution is rendered acid by addition of phosphoric acid.

Not only the simple or reserve proteins but also the so-called formative proteins, are broken down in the plant. When seeds germinate in darkness adenin, guanin, xanthin and hypoxanthin are produced, as cleavage products of nucleic acid. The studies of Karapetova and Sobashnikova,[4] who employed seedlings of rye and barley grown with inadequate nutrition, show that the proteins found to be indigestible in gastric juice are not as easily broken down in the plant as are the ones that are digestible in gastric juice. In the early stages of development the amount of indigestible proteins actually increases, while the total amount of protein decreases. Decomposition of the indigestible proteins occurs later. Zaliesskii[5] has also pointed out that nucleo-proteins are to be considered as formative (non-reserve) materials, on account of their relative stability as revealed by their behavior when the organism is in a starved condition. It may be supposed that those substances that are first decomposed during starvation are nutrient materials, while those remaining unchanged are constituents of the protoplasm. Pronounced decomposition of the nucleo-proteins is to be observed only in dead plants that still possess active enzymes.

The decomposition of formative proteins (nucleo-proteins and nucleo-albumins) may be estimated from the decrease in phosphorus-containing pro-

[1] **Godlewski, E.,** Nouvelle contribution à l'étude de la respiration intramoléculaire des plantes. [Title in Polish, German and French.] Bull. Int. Acad. Sci. Cracovie (Math.-nat. Cl.) (Anz. Akad. Wiss. Krakau.) 1904: 115–158. 1904.

[2] **Butkewitsch, Wl.,** Umwandlung der Eiweissstoffe durch die niederen Pilze im Zusammenhange mit einigen Bedingungen ihrer Entwickelung. Jahrb. wiss. Bot. 38: 147–240. 1903.

[3] **Butkevich,** 1903. [See note 2, this page.]

[4] **Karapétoff, H., and Sabachnikoff, M.,** Sur le décomposition des matières protéiques dans les plantes. Rev. gén. bot. 14: 483–486. 1902.

[5] **Zaleski, W.,** Ueber die Rolle der Nucleoproteide in den Pflanzen. Ber. Deutsch. Bot. Ges. 29: 146–155. 1911.

teins. Ivanov[1] determined the amounts of phosphorus of various kinds of compounds, in seeds and etiolated seedlings of *Vicia faba*, and obtained the results given in the table below, where the numbers are percentages, on the basis of total phosphorus content.

	Seeds	Seedlings 5 Days Old	Seedlings 20 Days Old
Phosphorus of inorganic phosphates	11.4	48.1	80.2
Phosphorus of lecithin	11.6	....	6.6
Phosphorus of proteins	52.5	37.4	13.7
Phosphorus of organic phosphates	25.7	9.8	5.1

Germination in darkness thus appears to be correlated with a pronounced decomposition of phosphorus-containing proteins. In resting seeds protein phosphorus amounted to 52.5 per cent. of the total phosphorus content, while seedlings twenty days old contained protein phosphorus amounting to only 13.7 per cent.; in the latter case most of the phosphorus occurred as inorganic phosphates.[2] Zaliesskii[3] obtained results similar to these. He found, for example, that the phosphorus-containing proteins disappear from the cotyledons of germinating seeds, while the amount of these proteins increases markedly in the axial organs, since growth is accompanied by synthetic processes. Thus, in the axial parts of *Vicia faba* seedlings three days old, the ratio of protein phosphorus to protein nitrogen was found to be 0.0125:0.0850(1:6.8), while the corresponding ratio for seedlings nine days old was 0.0337:0.3755 (1:11.1).

The researches of Burkevich, Zaliesskii,[4] Ivanov,[5] Kovshov[6] and Gromow[7] show that the cleavage of phosphorus-containing as well as that of phosphorus-free nitrogens is dependent upon enzymatic processes.

[1] **Ivanow, Leonid,** Ueber die Umwandlungen des Phosphors beim Keimen der Wicke. (Vorläufige Mittheilung.) Ber. Deutsch. Bot. Ges. **20**: 366–372. 1902. **Idem,** Ueber die Umwandlungen des Phosphors in der Pflanze im Zusammenhange mit der Eiweissstoffmetamorphose. Arbeit. Naturforscherges. St. Petersburg **34**: 1–170. 1905. [Russian.] [Rev. in: Bot. Centralbl. **103**: 83. 1906.]

[2] **Vorbrodt, Wlad.,** Untersuchungen über die Phosphorverbindungen in den Pflanzensamen, mit besonderer Berücksichtigung des Phytins. [Title also in Russian. Text in German.] Bull. internat. (classe sci. math. et nat., ser A.) Acad. Sci. Cracovie **1910**: 414–511. 1910.

[3] **Zaelski, W.,** Beiträge zur Verwandlung des Eiweissphosphors in den Pflanzen. Ber. Deutsch. Bot. Ges. **20**: 426—433. 1922. **Idem,** Ueber den Umsatz der Nucleinsaüre in keimenden Samen. *Ibid.*, **25**: 349–356. 1907.

[4] **Zaleski, W.,** Beiträge zur Kenntnis der Eiweissbildung in reifenden Samen. (Vorläufige Mitteilung.) Ber. Deutsch. Bot. Ges. **23**: 126–133. 1905. **Idem,** Zur Kenntnis der proteolytischen Enzyme der reifenden Samen. *Ibid.* **23**: 133–142. 1905. **Idem,** Ueber den Umsatz der Phosphorverbindungen in reifenden Samen. *Ibid.* **25**: 58–66. 1907. **Idem,** Ueber die autolytische Ammoniakbildung in den Pflanzen. *Ibid.* **25**:357–360. 1907. **Idem,** 1907. [See note 3, this page.]

[5] **Ivanov,** 1902, 1905. [See note 1, this page.]

[6] **Kovshov, I. D.,** Fermentative Eisweisszersetzung in erfrorenen Pflanzen. [Russian, with German abstract.] Trav. Soc. Imp. Nat. St.-Pétersbourg **35**[3] (Jour. bot. **1**): 180–185. 1906. [German abstract, p. 187.]

[7] **Gromow, T.,** and **Grigoriew, O.,** Die Arbeit der Zymase und der Endotryptase in den abgetöteten Hefezellen unter verschiedenen Verhältnissen. Zeitsch. physiol. Chem. **42**: 299–329. 1904.

§5. Nitrogenous Products of Protein Decomposition.—Asparagin (NH_2-CO—CH_2—$CHNH_2$) is the most important product of protein decomposition in plants. Germinated legumes that have beeen kept in the dark, especially *Lupinus luteus*, are notably rich in this substance. According to Borodin,[1] asparagin is not present in the Caryophyllaceæ, in which glutamin occurs, however. Glutamin (NH_2CO—CH_2—CH_2—$CHNH_2$—$COOH$) is a product similar to asparagin, but it is known only in isolated cases, since it is difficult to bring to crystallization and gives no definite reaction. This substance is present in sugar beets and is abundant in Curcurbita seedlings. It takes the place of asparagin in the Caryophyllaceæ and in ferns.[2]

The following amino acids and basic substances may be mentioned as other products of protein decomposition in plants.

MONOAMINO ACIDS

Leucin, $(CH_3)_2.CH$—CH_2—$CHNH_2$—$COOH$.
Tyrosin, C_6H_4OH—CH_2—$CHNH_2$—$COOH$.
Valin, $(CH_3)_2$—CH—$CHNH_2$—$COOH$.

BASIC SUBSTANCES[3]

Lysin, NH_2CH_2—CH_2—CH_2—CH_2—$CHNH_2$—$COOH$.

Arginin, $HN=C\begin{cases} NH_2 \\ NHCH_2—CH_2—CH_2—CHN_2H—COOH. \end{cases}$

Histidin, $\begin{array}{ccc} & CH & \\ NH & & N \\ | & & | \\ CH & & C—CH_2—CHNH_2—COOH. \end{array}$

Large amounts of arginin are present[4] in conifer seedlings. The purin bases,[n] xanthin, hypoxanthin, adenin and guanin, the formulas for which are given below, arise from the decomposition of the nucleo-proteins.

[1] **Borodin,** 1885. [See note 3, p. 172.]

[2] **Schulze, E.,** Ueber die Verbreitung des Glutamins in den Pflanzen. Landw. Versuchsst. 48: 33–55. 1897.

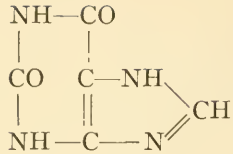
[3] **Schulze, E.,** and **Winterstein, E.,** Ueber die bei der Spaltung der Eiweisssubstanzen entstehenden basischen Produkte. Ergeb. Physiol. 1: 32–61. 1902.

[4] **Schulze, E.,** Ueber die beim Umsatz der Proteïnstoffe in den Keimpflanzen einiger Coniferen-Arten entstehenden Stickstoffverbindungen. Zeitsch. physiol. Chem. 22: 435–448. 1896–1897.

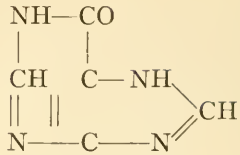
[n] The purin bases may be considered as derived from purin, which is not found in nature, but which has been synthetized. It may be represented as follows, the various atomic positions in the two rings being numbered.

$$\begin{array}{lllll} (1) & N = & (6) & CH & \\ & | & & | & \\ (2) & CH & (5) & C—(7)\ NH & \\ & \| & & \| & \quad\diagdown (8)CH \\ (3) & N— & (4) & C—(9)\ N & \quad\diagup \end{array}$$

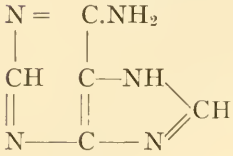
Referring to the numbers, xanthin is called 2-6-dioxypurin. Adenin is 6-aminopurin, hypoxanthin is 6-oxypurin, and guanin is 2-amino-oxypurin.—*Ed.*



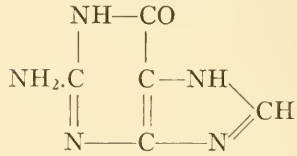
Xanthin



Hypoxanthin



Adenin



Guanin

Among these decomposition products are also the xanthin derivatives, caffein (1-3-7-trimethyl-xanthin) and theobromin (3-7-dimethyl-xanthin[1]).

Recent accounts of the formation of polypeptids in plants are very interesting.[2] These products may be either primary or secondary, the latter being formed by secondary synthetic processes. Tryosin and leucin are among the primary products and are formed by protein hydrolysis due to proteolytic enzymes. Asparagin, on the contrary, is a secondary product, arising through the transformation of primary products. Tyrosin and leucin, for example, occur only in the first stages of the development of seedlings of *Lupinus luteus*, while asparagin practically replaces these substances in later stages. The following analyses[3] of lupine seedlings fifteen and eighteen days old show the increase in asparagin content as the seedlings become older. [The values are percentages on the basis of the total dry weight of the seeds before germination.

	Seedlings 15 Days Old	Seedlings 18 Days Old
Nitrogen of proteins	1.49	1.51
Nitrogen of asparagin	3.85	4.23
Nitrogen of other compounds	1.27	0.77

Both groups of seedlings are seen to contain similar quantities of proteins, but the amount of asparagin in the older seedlings is greater that that in the younger ones. The increase in asparagin content arises at the expense of the lower decomposition products, the amount of which is seen to be correspondingly decreased.

The amino acids formed in the primary decomposition of proteins are further transformed without oxidation, and the transformation products thus produced have been called aporrhegmas.[4] Furthermore, methylation and splitting

[1] **Weevers, Th.**, Die physiologische Bedeutung des Kaffeins und des Theobromins. Ann. Jard. Bot. Buitenzorg *II*, **6**: 1–78. 1907.

[2] **Schulze, E.**, Neue Beiträge zur Kenntnis der Zusammensetzung und des Stoffwechsels der Keimpflanzen. Zeitsch. physiol. Chem. **47**: 507–569. 1906.

[3] **Merlis, M.**, Ueber die Zusammensetzung der Samen und der etiolierten Keimpflanzen von Lupinus augustifolius L. Landw. Versuchsst. **48**: 419–454. 1897.

[4] **Ackermann, D.**, and **Kutscher, Fr.**, Ueber die Aporrhegmen. Zeitsch. physio. Chem. **69**: 265–272. 1910. **Ackermann, D.**, Ueber ein neues, auf bakteriellem Wege gewinnbares, Aporrhegma. *Ibid.*, **69**: 273–281. 1910. **Engeland, R.**, and **Kutscher, Fr.**, Ueber ein methyliertes Aporrhegma des Tierkörpers. *Ibid.* **69**: 282–285. 1910.

by oxidation alter the composition of the aporrhegmas. The end product of these processes is ammonia, which is then used in the synthesis of asparagin.[1]

The following method is employed in the quantitative study of the various nitrogenous substances that have been mentioned in the preceding paragraphs.[2] The total nitrogen content is determined from one portion of the material, and the protein nitrogen is determined from an other portion, the difference between these two quantities being the amount of the non-protein nitrogen. For the determination of the separate nitrogen compounds, the plants to be studied are extracted with water and the extract is precipitated with lead acetate. The precipitate contains proteins, pigments and other compounds, while the crystalline nitrogenous substances are in the filtrate. The filtrate is treated with mercuric nitrate, which precipitates asparagin, glutamin and allantoin; also, in part, xanthin, hypoxanthin, guanin, arginin, tyrosin. The precipitate is suspended in water, treated with hydrogen sulphide and the mercuric sulphide thus formed is filtered out. The filtrate is neutralized with ammonia and concentrated by evaporation, after which it is allowed to stand for some time. Crystals of the nitrogenous compounds separate out and may be further dealt with by suitable methods. If no material is precipitated by mercuric nitrate, then the plant extract is treated with lead acetate and filtered, the filtrate being treated directly with hydrogen sulphide. The lead sulphide is filtered off, the filtrate is neutralized with ammonia and then concentrated by evaporation over a water bath.

The method of Sachsse[3] is used especially in the determination of asparagin and glutamin. This procedure depends upon the fact that these amides break down, upon being boiled with weak hydrochloric acid and water, into amino acids and ammonia, as is illustrated by the following equation.

$$\underset{\text{(Asparagin)}}{NH_2.CO.CH_2.CHNH_2.COOH} + H_2O = \underset{\text{(Aspartic acid)}}{COOH.CH_2.CHNH_2.COOH} + \underset{\text{(Ammonia)}}{NH_3}.$$

Half of the asparagin nitrogen is thus split off. The ammonia nitrogen is then determined, according to the usual methods, and the number thus obtained is doubled, to give the asparagin nitrogen. The same method is of course also available for the determination of glutamin nitrogen.

For microchemical identification of asparagin the method of Borodin[4] is employed. The sections to be studied are mounted in alcohol under a cover glass and the alcohol is allowed slowly to evaporate out at the margin of the cover. If asparagin is present it crystallizes during this process. The crystals

[1] **Butkewitsch, Wl.,** Das Ammoniak als Umwandlungsprodukt stickstoffhaltiger Stoffe in höheren Pflanzen. Biochem. Zeitsch. **16**: 411–452. 1909. **Prianischnikow, D.,** and **Schulow, J.,** Ueber die synthetische Asparaginbildung in den Pflanzen. Ber. Deutsch. Bot. Ges. **28**: 253–264. 1910.

[2] **Abderhalden,** Handbuch. [See note 1, p. 155.]

[3] **Sachsse, Robert,** Ueber eine Methode zur quantitativen Bestimmung des Aparagins. Jour. prakt. Chem., n. F. **6**: 118–127. 1873.

[4] **Borodin,** 1878. [See note 3, p. 171.]

are tested for solubility in a saturated solution of asparagin, which dissolves all crystals but those of this substance.[o]

§6. Protein Synthesis in Plants.[p]—It has already been stated (page 31) that the primary protein synthesis occurs in leaves. The nitrogen necessary for such synthesis is mainly derived from the soil, as nitrates. Investigations upon the distribution of nitrates[1] in the plant have shown that they reach the leaves through the water-conducting system. Nitrates are found in leaves only in exceedingly small amounts, however, or else they are entirely absent, and it is therefore suggested that a transformation of nitrates must take place in these organs. Schimper[2] has proved, moreover, that the transformation of nitrates in leaves is connected with the photosynthetic assimilation of carbon. Accumulation of nitrates occurs in plants that have been kept in darkness, and these salts are used up afterwards, when the plants are exposed to light. Also, in chlorotic leaves, which are incapable of photosynthesis, no transformation of nitrates occurs in the light. Experiments with variegated leaves are especially convincing in this connection. The green as well as the white parts of such leaves are filled with nitrates in the dark. After subsequent illumination only the green portions are found to be without nitrates; in the colorless parts the amount of nitrate remains unchanged.

From such experiments it has been concluded that protein synthesis in leaves occurs only in light. It must be noted, however, that in these experiments of Schimper a deficiency of carbohydrates surely occurred in the absence of light. This consideration is of great importance, since Zaliesskii[3] was able to demonstrate protein synthesis from carbohydrates and nitrates when darkened leaves were supplied with carbohydrates by means of a nutrient solution. It thus appears that protein synthesis in leaves is only indirectly dependent upon light. Only in light is the formation of carbohydrates possible, and these substances are necessary for the formation of proteins. It is quite possible, however, that with an adequate supply of carbohydrates, protein synthesis may go on more rapidly in light than in darkness.

[1] **Wulfert, H.**, Ueber die Bestimmung der Salpetersäure bei Gegenwart organischer Substanzen. Landw. Versuchsst. **12**: 164–184. 1869. **Montéverdé**, Arbeit. Naturforscherges St. Petersburg. 1882.* **Berthelot, [Marcellin]**, and **André, [Gustave]**, Sur l'existence et sur la formation des azotates dans le regne végétal. Ann. chim. et phys. *VI*, **8**: 5–8. 1886. **Idem**, Les azotates dans les végétaux. I. Méthodes 2'analyze. *Ibid. VI*, **8**: 8–25. 1886. **Idem**, same title. *II*. Leur presence universelle. *Ibid. VI*, **8**: d6–31. 1886. **Idem**, Les azotates dans les plantes aux diverses periodes de la végétation. Plante totale. *Ibid. VI*, **8**: 32–63. 1886. **Idem**, Les azotates dans les différentes parties des plantes. *Ibid. VI*, **8**: 64–115. 1886. **Idem**, Sur la formation de salpètre dans les végétaux. *Ibid. VI*, **8**: 116–128. 1886.—**Berthelot, [Marcelin]**, and **André, [Gustave]**, Recherches sur la vègètation. Sur les carbonates dans les plantes vivantes. Ann. chim. et phys. *VI*, **10**: 85–107. 1887. **Idem**, Recherches sur l'acide oxalique dans la végétation. I. Méthodes d'analyze. *Ibid. VI*, **10**: 289–308. 1887. **Idem**, same title. II. Etude de diverses plantes. *bid. VI*, **10**: 308–350. 1887. **Idem**, Sur une relation entre la formation de l'acide oxalique et celle des principes albuminoides dans certains végétaux. *Ibid. VI*, **10**: 350–353. 1887.

[2] **Schimper, A. F. W.**, Ueber Kalkoxalatbildung in den Laubblättern. Bot. Zeitg. **46**: 65–69, 81–89. 97–107, 113–123, 129–139, 145–153. 1888.

[3] **Zaleski, W.**, Die Bedingungen der Eiweisssynthese in Pflanzen, p. 53. 1900.* **Idem**, Zur Kenntniss der Eiweissbildung in den Pflanzen. Ber. Deutsch. Bot. Ges. **15**: 536–542. 1897.

[o] This method is very unsatisfactory for several reasons. For better methods see **Molisch**, 1913. [See note 1, p. 90.]—*Ed.*

[p] The section is numbered §7 in German; the numbering of the 7th Russian edition is here followed.—*Ed.*

Treub[1] has developed the hypothesis that hydrocyanic acid is an intermediate product in protein synthesis. It is well known that many leaves contain appreciable amounts of hydrocyanic acid (in the form of glucosides). With suitable treatment such leaves give a chemical test for this acid by becoming intensely blue, with the formation of Prussian blue (Fig. 87, *a*). If the leaves are left several days in darkness the hydrocyanic acid disappears completely, as is shown by the complete absence of Prussian blue after application of the test. The leaf shown in Fig. 87 was divided along the midrib and one portion (*a*) was subjected to the test, after which the remaining portion (*b*) was kept in the dark for a time, the test being finally applied to this part also, without the formation of any Prussian blue.[q]

Leaves that have thus been depleted of hydrocyanic acid again produce it in considerable quantity when supplied with nitrate and sugar in darkness, or when supplied with nitrate in light. Considerable amounts of hydrocyanic acid are also contained in axial organs (young bamboo sprouts).[2]

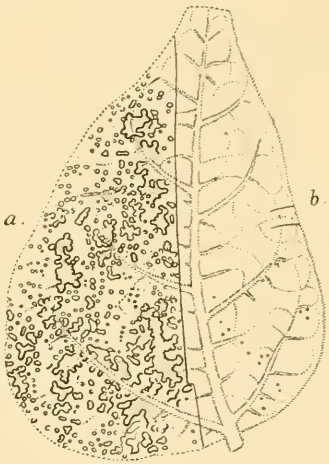


FIG. 87.—Leaf of *Phaseolus lunatus*, showing coloration with Prussian blue (*a*), due to presence of hydrocyanic acid.

Protein decomposition occurs in germinating seeds in darkness, while the later stages of germination in light exhibit protein synthesis. In this case also, light is directly necessary only for the formation of carbohydrates. Protein formation, out of carbohydrates and nitrogenous organic substances, is independent of light. Leek bulbs, for instance, contain little protein, but much carbohydrate and organic nitrogen. Consequently, according to Zaliesskii,[3] the sprouting of these bulbs in darkness is not accompanied by protein decomposition, but by its synthesis. The following data referring to leek bulbs in the dormant condition and after having grown for a month in darkness, may serve as an illustration of this. The numbers, excepting the last two, show the relative amounts of the various materials mentioned that were found in the two stages of development.

[1] **Treub, M.,** Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. Ann. Jard. Bot. Buitenzorg *II*, 4: 86–147. 1904. **Idem,** same title. *Ibid*, *II*, 6: 79–114. 1907.

[2] **Walther, O., Krasnosselsky, T. Maximow, N. A.,** and **Malcewsky, W.,** Ueber den Blausäuregehalt der Bambusschöszlinge. Bull. Déparetment Agric. Indes Néerlandaises, No. 42. 4 p. 1910.

[3] **Zaleski, W.,** Zur Keimung der Zweibel von Allium cepa und Eiweissbildung. (Vorläufige Mittheilung.) Ber. Deutsch. Bot. Ges. 16: 146–151. 1898.

[q] The test for hydrocyanic acid here referred to is carried out as follows: The leaf is punched full of minute holes by means of a bunch of fine needles and is then placed in 5 per cent. solution of potassium hydrate for a minute or two. It is then transferred to a warm (60°C.) aqueous solution of ferrous sulphate (2.5 per cent.) and ferric chloride (1 per cent.), where it remains about ten minutes. It is finally placed in hydrochloric acid (1 part of ordinary concentrated acid to 5 or 6 parts of water). The color develops after from five to fifteen minutes.—*Ed.*

	Dormant Bulbs	Sprouted Bulbs
Total dry weight	5.8246	4.7716
Total nitrogen	0.1614	0.1595
Nitrogen of protein	0.0517	0.0838
Nitrogen of substances precipitated by phosphotungstic acid	0.0252	0.0244
Nitrogen of asparagin	0.0121	0.0163
Nitrogen of other compounds	0.0744	0.0350
Protein nitrogen (percentage of total nitrogen)	32.0	52.5

Hettlinger[1] and Zaliesskii[2] showed also that a formation of protein is brought about by the wounding of onion bulbs and that this proceeds with considerable velocity. The same amount of protein was formed in four days after wounding as was found after a month of normal sprouting in darkness. The sprouted bulb was cut into four equal parts, one part being dried and the three others being left in darkness for four days. Analysis showed that the protein nitrogen of the dried portion amounted to 32.0 per cent. of the total nitrogen, while the corresponding percentages for the other portions were from 49.4 to 51.8.

Hansteen[3] showed that various nitrogenous substances are suited to the formation of protein. Zaliesskii and Kovshov[4] showed that protein formation in wounded onion bulbs occurs only in the presence of oxygen. According to Zaliesskii,[5] the process of protein transformation is altered when the surrounding atmosphere contains ether vapor. He showed that the axial organs of *Lupinus augustifolius*, when the cotyledons had been removed, were able to carry on protein synthesis in darkness if supplied with carbohydrates and nitrogen by means of a nutrient solution, and that this process was accelerated by the presence of ether vapor.

Present knowledge regarding the formation of nucleins in plants is very incomplete. It is well known that growth is generally accompanied by nuclein synthesis. Although the total protein content decreases during the germination of seeds in darkness, nevertheless the nucleo-proteins increase during the first stages of germination.[6] Fig. 88 shows that the germination of wheat in darkness is correlated with an increase in proteins indigestible in gastric juice, the amount of which is nearly proportional to the amount of nucleo-proteins.

Wounding produces increased vital activity. The work of Kovshov[7] shows that the formation of protein is accelerated in wounded onions. This increased synthesis results mainly in proteins indigestible in gastric juice, but there is no

[1] Hettlinger, A., Influence des blessures sur la formation des matières protéiques dans les plantes. Rev. gén. bot. 13: 248–250. 1901.

[2] Zaleski, W., Beiträge zur Kenntniss der Eiweissbildung in den Pflanzen. Ber. Deutsch. Bot. Ges. 19: 331–339. 1901.

[3] Hansteen, Barthold, Ueber Eiweisssynthese in grunen Phanerogamen. Jahrb. wiss. Bot. 33: 417–486. 1899.

[4] Kovchoff, J., L'influence des blessures sur la formation des matières protéiques non digestibles dans les plantes. Rev. gén. bot. 14: 449–462. 1902.

[5] Zaleski, W., Zur Aetherwirkung auf die Stoffumwandlung in den Pflanzen. (Vorläufige Mittheilung.) Ber. Deutsch. Bot. Ges. 18: 292–296. 1900.

[6] Palladin, W., Recherches sur la corrélation entre la respiration des plantes et les substances azotées actives. Rev. gén. bot. 8: 225–248. 1896. Zaliesskii, 1907. [See note 3, p. 174.]

[7] Kovchoff, 1902. [See note 4, this page.]

corresponding increase in the amount of protein phosphorus in the tissues, and it appears that the observed increase in these indigestible proteins is mainly made up of phosphorus-free compounds.[1]

In leaves the formation of proteins indigestible in gastric juice is dependent upon the presence of carbohydrates and upon illumination. Palladin[2] found that such indigestible proteins increase in etiolated bean leaves when these are supplied with saccharose, more of these proteins being formed in light than in darkness. The following table presents the results of two experiments in this connection, the numbers representing milligrams.

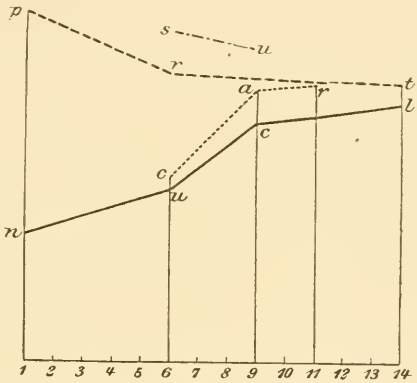


FIG. 88.—Graphs showing metabolic changes during germination of wheat seeds in darkness. *n-u-c-l*, indigestible protein content; *p-r-t*, total protein content; *c-a-r*, carbon dioxide eliminated; *s-u*, sugar content.

NITROGEN OF PROTEINS INDIGESTIBLE IN GASTRIC JUICE, CONTAINED IN 100 G. OF ETIOLATED LEAVES

	After Six Days on Saccharose Solution	
Freshly Gathered	in Darkness	in Light
18.6	82.6	166.4
18.6	51.9	115.4

§7. Alkaloids, Toxins and Antitoxins.[r]—Plants often contain various poisonous substances,[3] among which alkaloids and some glucosides are especially

[1] **Kovchoff, J.,** Ueber den Einfluss von Verwundlungen auf Bildung von Nucleoproteïden in den Pflanzen. Ber. Deutsch. Bot. Ges. 21: 165–175. 1903. **Zaleski, W.,** Ueber den Umsatz der Nucleinsäure in Keimenden Samen. *Ibid.* 25: 349–356. 1907. **Idem,** Ueber den Aufbau der Eiweissstoffe in den Pflanzen. *Ibid.* 25: 360–367. 1907. **Idem,** 1907 (Ammoniakbildung). [See note 4, p. 174.] **Ivanov,** 1902, 1905. [See note 1, p. 174.]

[2] **Palladin, W.,** Influence de la lumière sur la formation des matières protéiques actives et sur l'énergie de la respiration des parties vertes des végétaux. Rev. gén. bot. 11: 81–105. 1899.

[3] **Gauthier, A.,** Les toxines microbiennes et animals. Paris, 1896. **Brühl, Julius,** Die Pflanzenalkaloide. Braunschweig, 1900. **Rijnn, J. J. L. van,** Die Glykoside; Chemische Monographie der Pflanzenglykoside nebst systematischer Darstellung der künstlichen Glykoside. Berlin, 1900. **Winterstein, Ernst,** and **Trier, Georg,** Die Alkaloide, eine Monographie der natürlichen Basen. Berlin, 1910. **Faust,** Die tierischen Gifte. 248. p. Braunschweig, 1906.

[r] This section is numbered §4 in the German; the numbering of the 7th Russian edition is here followed.—*Ed.*

worthy of note. These poisons may be effective as accelerators of material exchange. According to Votchal,[1] solanin, which is a very poisonous alkaloid, is formed in various parts of the potato tuber, especially during the period of active growth. When the tuber is wounded a considerable amount of the solanin accumulates in the neighborhood of the wound. It will be shown later that respiration, as well as other metabolic processes, is increased by injury of plant tissues. Solanin thus seems to be a stimulant that increases metabolism in wounded regions.

Extremely active poisons are formed by bacteria. These organisms not only destroy dead bodies, but many of them infest even living plants and animals, thus giving rise to various infectious diseases. They are the so-called pathogenic forms. *Bacillus tetani*, the form that produces the disease known as tetanus or lockjaw, is a typical example of the anaerobic pathogenic bacteria, which develop only in the absence of oxygen. Many other pathogenic bacteria are aerobic, however, and attain their full development only in the presence of oxygen. *Bacillus anthracis*, which produces splenic fever, belongs in the latter group. It was by the study of anthrax that Pasteur first made the discovery that infectious diseases are caused and propagated by bacteria. It had long been known that many bacteria are present in the blood of animals suffering from splenic fever. Pasteur placed a drop of such blood in broth and obtained an abundant development of bacteria. Re-inoculations were made, from the first culture to a second, from the second to a third, etc., and the twentieth culture was still capable of producing the disease when an animal was infected with the liquid. Pasteur deserves credit also for working out the method of immunization by vaccination. In 1879 he began his work on the bacillus of chicken cholera. Pure cultures of this organism proved to have become greatly weakened by standing in a thermostat during the summer; inoculation therefrom produced only a local effect and failed to cause the death of the fowl. It also became evident that subsequent inoculation with extremely virulent, fresh cultures was without fatal effect if the fowls had previously been inoculated with the weakened culture. Generalizing from these observations, Pasteur arrived at vaccination as a protection against anthrax. He found that the virulence of the anthrax bacillus becomes weakened under the influence of high temperature, gradually losing its poisonous properties at from 42 to 43°C. Animals inoculated with such weakened cultures endure this inoculation, and are then no longer susceptible to injury from inoculation with stronger cultures; they are thus protected against anthrax. This leads to the supposition that toxins are neutralized by antitoxins that are produced in the animal tissues. A number of such antitoxins have been actually isolated. Vaccination protects against infection, but after the disease is already developed it may be controlled by direct injection of antitoxin. As is well known, diphtheria is combated by means of diphtheria antitoxin, which is obtained from the blood serum of horses

[1] **Votchal, E.**, Zur Frage von der Verbreitung, Vertheilung und Rolle des Solanins in den Pflanzen. II. Das Geschick des Solanins in der Pflanze and seine Bedeutung für das Leben derselben. [Title in Russian and German, article in Russian.] Trav. Soc. Nat. Univ. Imp. Kazan. **195**: 1–74. 1889. **Clautriau, G.**, Nature et signification des alcoloïdes végétaux. Rec. Inst. Bot. Bruxelles **5**: 1–87. 1902.

that have been previously immunized by vaccination. This method of treatment of diseases is called serumtherapy.

In many cases the pathogenic bacteria are distributed throughout the whole body of the infected animal or human being; in other cases they are localized in some special region. The bacilli of diphtheria and tetanus are thus localized. In such cases the injurious action of the bacteria is manifestly not directly due to their number but to their poisonous excretions. Although diphtheria bacilli develop only in the throats of human beings, nevertheless the entire body is poisoned by the toxins excreted by the bacterial cells. Diphtheria toxin may be obtained from bouillon cultures of the diphtheria bacillus by filtering the liquid through a Chamberland filter, the filtrate being very poisonous. Tetanus bacilli are present in many soils. If a wound is infected with tetanus, the bacteria develop only in the immediate neighborhood of the lesion but, even so, the disease is deadly, since tetanus toxin is extraordinarily poisonous. One gram of this toxin is capable of bringing about the death, by poisoning, of 75,000 men.

§8. Lipoids and Phosphatides.[8]—The term "lipoid," which was introduced by Overton,[1] may be understood to include[2] all tissue and cell constituents that may be extracted by ether and similar solvents. Here belong not only fats and fatty acids but also various other substances, among which cholesterin and complex phosphatides are especially important. Thudichum[3] designates as phosphatides those organic compounds containing phosphorus, that are soluble in alcohol and ether. These substances are very unstable and are chemically very active; they constitute an indispensable part of the protoplasm of all living cells. Many complex phosphatides undergo auto-oxidation.

.Recent investigation shows that phosphorus is not the only mineral substance contained in lipoids. Thus, Glikin[4] found that half of the total iron content of human and cow's milk is contained in lipoids. Winterstein and Stegmann[5] have found, in the leaves of Ricinus (castor bean), a phosphatide that contains 6.74 per cent. of calcium. Phosphatides containing carbohydrates are present in some plants.[6] It may be suggested that lipoids form combinations

[1] **Overton, Ernst,** Studien über die Narkose, zugleich ein Beitrag zur allgemeinen Pharmakologie. Jena, 1901.

[2] **Bang, Ivar,** Biochemie der Zellipoide. Ergeb. Physiol. 6: 131–186. 1907.

[3] **Thudichum, John L. W.,** Die chemische Konstitution des Gehirns des Menschen und der Tiere. Tübingen, 1901.

[4] **Glikin, W.,** Zur biologischen Bedeutung des Lecithins. III. Mitteilung. Ueber den Lecithin- und Eisengehalt in der Kuh- und Frauenmilch. Biochem. Zeitsch. 21: 348–354. 1909.

[5] **Winterstein, E.,** and **Stegmann, L.,** Ueber einen eigenartigen phosphorhaltigen Bestandteil der Blätter von Ricinus. VI. Mitteilung. Ueber Phosphatide. Zeitsch. physiol. Chem. 58: 527–528. 1908–1909.

[6] **Hiestand, O.,** Historische Entwickelung unserer Kenntnisse über die Phosphatide. Beiträge zur Kenntnis der pflanzlichen Phosphatide. Zürich, 1906.[4] **Winterstein, E.,** and **Hiestand, O.,** Beiträge zur Kenntnis der pflanzlichen Phosphatide. II. Mitteilung. Zeitsch. physiol. Chem. 54: 288–330. 1907–08. **Winterstein, E.,** Beiträge zur Kenntnis pflanzlicher Phosphatide. III. Mitteilung. *Ibid.* 58: 500–505. 1908–09. **Winterstein, E.,** and **Smolenski, K.,** Beiträge zur Kenntnis der aus Cerealien darstellbaren Phosphatide. IV. Mitteilung. Ueber Phosphatide. *Ibid.* 58: 506–521. 1909. **Smolenski, K.,** Zur Kenntnis der aus Weizenkeimen darstellbaren Phosphatide. V. Mitteilung. Ueber Phosphatide. *Ibid.* 58: 522–526. 1908–09.

[8] For a recent discussion of these substances see: **Rosenbloom, Jacob,** and **Gies, W. J.,** A proposed chemical classification of the lipins, with a note on the intimate relation between cholesterols and bile salts. Biochem. bull. 1: 51–56. 1911. **Rosenbloom, J.,** Intracellular lipins. *Ibid.* 1: 75–79. 1912.—*Ed.*

with proteins, in plants as well as in animals, the labile, complex substances thus produced being split up by hot alcohol. The results of Bondi and Eissler[1] support this suggestion. They obtained lipoid-proteins soluble in alcohol, by the linking together of fatty acids and amino acids; these substances are broken down by hydrolyzing enzymes. Since the chemical composition of lipoids is very complex and since they show marked adsorption phenomena, no reliable method for the isolation of lipoids and phosphatides is as yet available.[2] Nevertheless numerous investigations already show that lipoids play an extremely important rôle in the activity of the cell.[3] Studies upon the distribution of lipoids as determined microchemically have been carried out by Ciaccio.[4] Kossel[5] states that lecithin is always present in every protoplast. The extended researches of Schulze[6] and his school, and those of Stoklasa[7] and other authors, have demonstrated beyond question that phosphatides are widely distributed in plants. According to Stoklasa, lecithin accompanies proteins in plants, and seeds rich in protein also contain an appreciable amount of phosphatides. The relative protein, phosphatide and fat contents of various seeds are shown below.

Kind of Seed	Protein	Phosphatides	Fats
Lupinus luteus (yellow lupine).....	38.25	1.59	4.38
Pisum sativum (pea).....	23.13	1.23	1.89
Cannabis sativa (hemp).....	18.23	0.88	32.58
Helianthus annuus (sunflower).....	14.22	0.44	32.26
Zea mais (maize).....	9.12	0.28	4.36

The researches of Palladin and Stanevich[8] show that plant respiration is dependent upon lipoids. Wheat seedlings were treated with various solvents, toluol, benzene, acetone, benzine, turpentine, chloroform, ether, alcohol. The greater the amount of lipoids extracted, the smaller was the quantity of

[1] **Bondi, S.,** and **Eissler, Franz,** Ueber Lipoproteide und die Deutung der degenerativen Zellverfettung. VI. Weitere Spaltungsversuche mit Lipopeptiden. Biochem. Zeitsch. **23**: 510–513. 1910.

[2] **Schulze, E.,** and **Winterstein, E.,** Phosphatide. In: **Abderhalden,** Handbuch **2**: 256. 1909. [See note 1, p. 155.]

[3] **Bang, Ivar,** Biochemie der Zellipoide II. Ergeb. Physiol. **8**: 463–523. 1909.

[4] **Ciaccio, Carmelo,** Ueber das Vorkommen von Lecithin in den zellularen Entzündungsprodukten und über besondere lipoidbildende Zellen (Lecithinzellen). Centrlbl. allg. Pathol. u. pathol. Anat. **20**: 385–390. 1909.

[5] **Kossel, A.,** Chemische Zusammensetzung der Zelle. Arch. Physiol. **1891**: 181–186. 1891. (Review by **Sachsse** in Chem. Centralbl. 62^{III}: 37–38. 1891.)

[6] **Schulze E.,** and **Steiger, E.,** Ueber den Lecithingehalt der Pflanzensamen. Zeitsch. physiol. Chem. **13**: 365–384. 1889. **Schulze, E.,** and **Likiernik, A.,** Ueber das Lecithin der Pflanzensamen. *Ibid.* **15**: 405–414. 1891. **Schulze, E.,** and **Winterstein, E.,** Beiträge zur Kenntnis der aus Pflanzen darstellbaren Lecithine. (Erste Mitteilung.) *Ibid.* **40**: 101–119. 1903–04. **Schulze, E.,** and **Frankfurt, S.,** Ueber den Lecithingshalt einiger vegetabilischen Substanzen. Landw. Versuchsst. **43**: 307–318. 1894.

[7] **Stoklasa, Julius,** Die Assimilation des Lecithins durch die Pflanze. Sitzungsber. (math.-naturw. Kl.) K. A kad. Wiss. Wien 104^{I}: 712–722. 1895. **Idem,** Ueber die Entstehung und Umwandlung des Lecithins in der Pflanze. Zeitsch. physiol. Chem. **25**: 398–405. 1898.

[8] **Palladin, W.,** and **Stanewitsch, E.**. Die Abhängigkeit der Pflanzenatmung von den Lipoiden. Biochem. Zeitsch. **26**: 351–369. 1910.

carbon dioxide formed. Korsakova[1] has shown that lipoids likewise influence the activity of proteolytic enzymes.

Among the phosphatides, phytin[2] is especially noteworthy; it probably represents the first product in the assimilation of phosphoric acid.

§9. **Carbohydrates.**—The carbohydrates cellulose and starch are especially widely distributed in plants. Anatomical observation shows that the growth of cell walls and the formation of starch grains occur only in the immediate presence of protoplasm or leucoplasts. Starch and cellulose thus appear to be transformation products of proteins.[3] Physiological studies also support this supposition. The formation of starch and cellulose is accompanied by a decomposition of proteins, whereby nitrogenous compounds, especially asparagin, are formed. Thus, for example, the experiments of Hungerbühler[4] upon ripening potatoes gave the following results, which show that starch formation is accompanied by splitting of proteins and a formation of nitrogenous decomposition products.

Date of Test	Starch, Per Cent. of Total Dry Weight	Protein Nitrogen, Per Cent. of Total N	Non-protein Nitrogen, Per Cent. of Total N
June 23	56.7	70.9	29.1
June 30	61.3	64.4	35.6
July 7	66.3	58.7	41.3

On theoretical grounds Palladin[5] supposes that the formation of cell walls and of starch grains is accompanied by oxygen absorption, a supposition that is supported by anatomical observations.

Starch, which is insoluble in water, acts as a reserve material, the cells being frequently quite filled with it. If this reserve material were stored as water-soluble compounds (such as glucose), the cell walls would not then be able to withstand the enormous osmotic pressures that would develop.

The cell wall was long considered as made up of a single substance and as having a simple structure, but it was later shown that it is complex. Schulze[6] has classified the cell-wall constituents into two groups. The first group includes hemicelluloses, which can be extracted by heating in a 1-per cent. solution of hydrochloric or sulphuric acid. Among these substances is paragalactan, which is insoluble in water and is transformed by oxidation into mucic acid; it

[1] **Korsakow, Marie,** Ueber den Einfluss der Zelllipoide auf die Autolyse der Weizenkeime. Biochem. Zeitsch. 28: 121–126. 1910.

[2] **Vorbrodt,** 1910. [See note 2, p. 174.]

[3] **Langstein, Leo,** Die Bildung von Kohlehydraten aud Eiweiss. Ergeb. Physiol. 1: 63–109. 1902.

[4] **Hungerbühler, J.,** Zur Kenntniss der Zusammensetzung nicht ausgereifter Kartoffelknollen. Landw. Versuchsst. 32: 381–388. 1886.

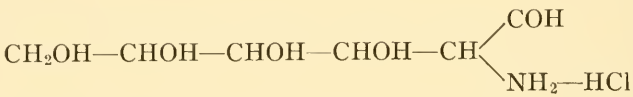
[5] **Palladin, W.,** Kohlehydrate als Oxydationsproducte der Eiweissstoffe. Ber. Deutsch. Bot. Ges. 7: 126–130. 1889.

[6] **Schulze, E., Steiger, E., and Maxwell, W.,** Zur Chemie der Pflanzenzellmembranen. (I. Abhandlung.) Zeitsch. physiol. Chem. 14: 227–273. 1890. **Schulze, E.,** Zur Chemie der pflanzlichen Zellmembranen. (II. Abhandlung.) *Ibid.* 16: 387–438. 1892. **Idem,** Zur Chemie der pflanzlichen Zellmembranen. (III. Abhandlung.) *Ibid.* 19: 38–69. 1894.

forms galactose by hydrolysis. Other hemicelluloses of the cell wall are hydrolyzed to mannose, arabinose, and xylose. The second group of cell-wall constituents contains the true celluloses, which do not go into solution on being warmed with 1-per cent. acid. By hydrolysis they produce glucose only, and by oxidation they give saccharic acid.

Cellulose does not always serve only as mechanical support; in many seeds thickenings of the cell walls are simply reserve materials, which are resorbed during germination.[1] This reserve cellulose consists of hemicelluloses, especially of mannans and galactans.[t]

The cell walls of many fungi differ from those of other plants in that they contain nitrogen. Those of *Boletus edulis*, *Agaricus campestris*, *Morchella esculenta*, *Botrytis cinerea*, and *Polyporus officinalis* furnish illustrations of this characteristic. The nitrogen content may be as great as 5.5 per cent.[2] If the cell walls of such fungi are hydrolyzed by heating with hydrochloric acid, glucosamin chlorhydrate is obtained as a decomposition product. It is represented as follows:



The same substance results from the hydrolysis of the chitin of insects. The cell walls of fungi thus contain substances that are very similar to chitin.

Grape sugar (glucose) is present in many active cells,[3] and therefore merits particular attention, especially since it is one of the simplest carbohydrates. The structural formula of dextro-glucose, or dextrose (which, in solution, rotates the plane of polarized light to the right) is as follows:

$$CHO—HCOH—HOCH—HCOH—HCOH—CH_2OH.$$

Cane sugar (saccharose) was formerly considered to be of limited distribution. With refinement of methods,[4] however, considerable amounts of this sugar have been found in growing organs.[5] Brown and Morris[6] have identified cane sugar in leaves and consider it to be the first product of the photosynthetic assimilation of carbon dioxide.[u] Only after the accumulation of considerable amounts of

[1] **Elfert, Th.**, Ueber die Auflöspungsweise der sekundären Zellmembranen der Samen bei ihrer Keimung. Bibliotheca botanica, Heft. 30, viii+26 p. Stuttgart, 1894.

[2] **Winterstein, E.**, Ueber ein stickstoffhaltiges Spaltungsproduct der Pilzcellulose. Ber. Deutsch. Chem. Ges. 27^{III}: 3113–3115. 1894. **Idem**, Ueber die Spaltungsproducte der Pilzcellulose. *Ibid.* 28^{I}: 167–169. 1895.

[3] **Armstrong, E. F.** The simple carbohydrates and glucosides. London, 1910.

[4] **Schulze, E.**, Ueber den Nachweis von Rohrzucker in vegetabilischen Substanzen. Landw. Versuchsst. 34: 408–413. 1887. **Schulze, E.**, and **Frankfurt, S.**, Ueber die Verbreitung des Rohrzuckers in den Pflanzen, über seine physiologische Rolle und über lösliche Kohlenhydrate, die ihn begleiten. Zeitsch. physiol. Chem. 20: 511–555. 1895.

[5] **Seliwanoff, Th.**, Ein Beitrag zur Kenntnis der Zusammensetzung etiolierter Kartoffelkeime. Landw. Versuchsst. 34: 414–417. 1887. **Frankfurt, Solomon**, Ueber die Zusammensetzung der Samen und der etiolierten Keimpflanzen von Cannabis sativa und Helianthus annuus. *Ibid.* 43: 143–182. 1894.

[6] **Brown** and **Morris**, 1893. [See note 1, p. 28.]

[t] This sentence is omitted in the 7th Russian edition.—*Ed.*

[u] On this point, however, see: **Dixon, H. H.**, and **Mason, T. G.**, The primary sugar of photosynthesis. Nature **97**: 160. 1916.—*Ed.*

cane sugar is there any transformation of the latter into starch; Böhm obtained quite analogous results by artificially supplying sugar to the plant.

§10. Glucosides.[v]—Glucosides[1] are chemical combinations of glucose (sometimes of other sugars) with various other substances, and they are split into their component parts by the action of acids or glucoside-splitting enzymes. For example, under the influence of emulsin, arbutin takes up water and produces hydroquinone and glucose. This reaction is shown below:[w]

$$\overset{\overset{\text{O}}{\text{———}}}{\text{CH}_2\text{OH—CHOH—CH—CHOH—CHOH—CH—OC}_6\text{H}_4\text{OH}} \text{ (arbutin)} +$$

$$\text{H}_2\text{O (water)} = \text{CH}_2\text{OH—CHOH—}\overset{\overset{\text{O}}{\text{———}}}{\text{CH—CHOH—CHOH—CHOH}} \text{ (glucose)} +$$

$$\text{HOC}_6\text{H}_4\text{OH (hydroquinone).}$$

Indican, a glucoside of the indigo plant, etc., forms glucose and indoxyl, with the taking up of water:

$$\text{C}_7\text{H}_6\text{NC—O—C}_6\text{H}_{11}\text{O}_5 \text{ (indican)} + \text{H}_2\text{O (water =}$$

$$\text{C}_6\text{H}_{12}\text{O}_6 \text{ (glucose)} + \text{C}_6\text{H}_4\left\langle\begin{matrix}\text{COH}\\ \\ \text{NH}\end{matrix}\right\rangle\!\!=\text{CH (indoxyl).}$$

Indoxyl oxidizes in the air, forming dark blue indigotin (indigo blue) and water:

$$2\text{C}_8\text{H}_7\text{ON} + \text{O}_2 = 2\text{H}_2\text{O} + \text{C}_{16}\text{H}_{10}\text{O}_2\text{N}_2.$$

Indigotin has the structural formula,

$$\text{C}_6\text{H}_4\left\langle\begin{matrix}\text{CO}\\ \\ \text{NH}\end{matrix}\right\rangle\text{C}=\text{C}\left\langle\begin{matrix}\text{CO}\\ \\ \text{NH}\end{matrix}\right\rangle\text{C}_6\text{H}_4.$$

As a third example may be mentioned amygdalin, an α-β glucoside of almond, peach, etc., which takes up water and splits into glucose, benzaldehyde and hydrocyanic acid:

$$\text{CH}_2\text{OH—CHOH—CH—}\overset{\overset{\text{O}}{\text{———}}}{\text{CHOH—CHOH—CH}}\text{—O—CH}_2\text{—CHOH—}$$

$$\text{CH—}\underset{\underset{\text{O}}{\text{———}}}{\text{CHOH—CHOH—CH}}\text{—O—CH}\overset{\overset{\text{C}_6\text{H}_5}{|}}{\underset{\underset{\text{CN}}{|}}{}} \text{ (amygdalin)} + \text{H}_2\text{O (water)} = 2\text{C}_6\text{H}_{12}\text{O}_6$$

(glucose) + C_6H_5—CHO (benzaldehyde) + HCN (hydrocyanic acid).

Glucosides may undergo autolysis in the tissues. Thus, if leaves of *Polygonum tinctorium* are exposed to an atmosphere saturated with chloroform vapor (which kills the cells), blue indigotin is formed in the tissues. The chlorophyll may then be extracted by alcohol, leaving the leaves blue. When

[1] **Rijn, van,** 1900. [See note 2, p. 333.]

[v] This section appears for the first time in the 7th Russian edition.—*Ed.*

[w] For this and similar statements of formulas and reactions, see **Haas** and **Hill,** 1913. [See note 3, p. 6.] Also see works on organic chemistry; an excellent short treatise for physiological students is **Bernthsen, A.,** A text-book of organic chemistry. Translated and edited by J. J. Sudborough. New York. 1907.—*Ed.*

autolysis occurs in plant parts containing amygdalin, a strong odor of hydrocyanic acid is developed.

Some of the glucosides that accumulate in plants appear to be respiratory chromogens, others are very efficient activators (hormones).

§11. Organic Acids.[x]—All living cells always contain some organic acids, the cell sap always giving an acid reaction. It is supposed that these acids arise through incomplete oxidation of carbohydrates. Numerous studies have been carried out upon oxalic acid in the form of its calcium salt,[1] and it appears that marked accumulation of this salt occurs in most plants only in light and with normal or high transpiration, while very little is formed in darkness and when transpiration is low.

Various external and internal conditions have great influence upon the formation and decomposition of organic acids in plants.[2] The amounts of these acids decrease somewhat in light, as is shown by the table below, which presents the relative acid contents of several plants, in darkness and in light.

Plant	Relative Acid Content In Darkness	In Light
Convallaria majalis (rhizome)	72	68
Phaseolus multiflorus (roots)	69	64
Etiolated wheat seedlings	238	230

The acid content is lower with higher temperatures. Thus, for example, plants of *Sempervivum tectorum*, with an acid content of 358, were placed in diffuse light for three hours, with temperatures of 4–6°C., 22–25°C., and 35–38°C., and at the end of this period the acid content had fallen to 336, to 327, and to 301, respectively.

If carbohydrates are artificially supplied, an increase in the acid content occurs. The roots were removed from etiolated seedlings of Phaseolus and some were placed in distilled water, others in a solution of glucose, in darkness. After three days the acid content of those on water was 185, while that of the plants in glucose solution was 257. Grape sugar thus produces an increase in the acid content of seedlings.

[Active roots appear to give off organic acids, into the soil, when the supply of oxygen is low. With a plentiful supply of oxygen they appear to give off only carbon dioxide.]

§12. The Importance of Water in Plants.[y]—Physiological processes can-

[1] **Kohl, Friedrich Georg,** Anatomisch-physiologische Untersuchung der Kalksalze und Kieselsäure in der Pflanze. Ein Beitrag zur Kenntnis der Mineralstoffe im lebenden Pflanzenkörper Marburg, 1889. **Monteverde, N. A.,** On the deposition of the oxalates of calcium and magnesium in plants. [Russian.] 81 p. St. Petersburg, 1889. Rev. in Bot. Centralbl. 43: 327–333, 1890. **Wehmer, Carl,** Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. Bot. Zeitg. 49: 233–246, 249–257, 271–280, 289–298, 305–313, 321–332, 337–346, 353–363, 369–374, 385–396, 401–407, 417–428, 433–439, 449–456, 465–478, 511–518, 531–539, 547–554, 563–569, 579–584, 596–602, 611–620, 630–638. 1891.

[2] **Puriewitsch Konstantin A.,** Bildung und Zersetzung der organischen Säuren in Samenpflanzen. Kiev, 1893.

[x] This section is numbered §10 in the German; the numbering of the 7th Russian edition is here followed.—*Ed.*

[y] This section appears for the first time in the 7th Russian edition.—*Ed.*

not go on without water in the cells.[1] About 80 or 90 per cent. (by weight) of the active plant cell is water, and the water content is small only in so-called resting tissues, such as those of dry seeds. When such relatively inactive tissues become active, the acceleration of the physiological processes is preceded or accompanied by pronounced absorption of water. At the same time much of the insoluble material of the inactive cells (starch, oil, etc.) is modified so as to become soluble in water, and this dissolves with the advance of renewed activity. Also, with the entrance of water many colloidal substances in the cell (which are not, or do not become, truly soluble in water) absorb this liquid to a marked degree and swell to a corresponding extent, even becoming so completely dispersed in the water that the *hydrosol* thus formed becomes liquid and assumes many of the properties of a true solution. In nearly dry cells these cell colloids are largely in the *hydrogel* phase and are virtually solid.

As the cell colloids pass into the sol phase and the crystalloids dissolve, those of the latter that are electrolytes become increasingly dissociated, so that they become much more active chemically. The water also is dissociated and a cell well supplied with water thus contains many different kinds of kations and anions, the concentrations of which determine the rates and directions of many chemical changes. Especially are the hydrogen ion (kation) and hydroxyl ion (anion) concentrations important in this way.[2]

Aside from being the medium of solution and dispersion of the non-aqueous cell substances, and aside from its influence on ionization and chemical action, water is also an essential *material* in the synthesis of organic compounds. The hydrogen and oxygen of the plant body are to be considered as derived from water (see Part I, Chapter I). Water is also a necessary material for the hydrolysis of many complex carbohydrates, proteins, fats, etc., into simpler compounds (*e.g.*, starch and cellulose into sugar, cane sugar into glucose). Of course water is produced by the opposite process (*e.g.*, the polymerization of glucose to form cane sugar), and also by the complete oxidation of carbohydrates, fats, etc., in respiration. But water apparently disappears in the earlier chemical steps of respiration (see page 225 and compare page 217).

§13. The Germination of Seeds.[2]—In the above discussion, some of the questions concerning various material changes and other physiological processes

[1] **Kraus, Gregor,** Ueber die Wasserverteilung in der Pflanze. Halle, 1879–1884. [This vol. is reprinted from Naturforscherges Halle; Festschr. (1879), 71 p.; Abhandl. 15: 49–120 (1880); 15: 229–319 (1881); 16: 141–205 (1884).] **Babcock, S. M.,** Metabolic water: its production and rôle in vital phenomena. Univ. Wisconsin Agric. Exp. Sta. Research Bull. 22. 1912. (Also Ann., rept. Wisconsin Agric. Exp. Sta. 292: 87–181. 1912.)

[2] [The true acidity" of a solution depends, not upon the total quantity of acid present, but upon the concentration of hydrogen ions; similarly, the "true alkalinity" depends upon the concentration of hydroxyl ions. Which concentration is in excess determines the reaction of the solution. It is necessary to remember that ion concentrations may be different in different parts of the same cell; for example, the protoplasm is generally alkaline while the cell-sap is acid. On the reactions of cell solutions, see: **Michaelis, L.,** Die allgemeine Bedeutung der Wasserstoffionenkonzentration für die Biologie. In **Oppenheimer's** Handbuch der Biochemie des Menschen und der Tiere. Jena, 1909–11. Ergänzungsband, 1913. (See Ergänzbd., p. 10.) **Sörensen, S. P. L.,** Ueber die Messung und Bedeutung der Wasserstoffionenkonzentration bei biologischen Prozessen. Ergeb. Physiol. 12: 393–532. 1912.]

[2] This section is numbered §11 in the German edition. It appears unnumbered, at the end of the chapter, in the 7th Russian edition.—*Ed.*

have been considered with reference to changes that occur in germinating seeds. The important factors in seed germination will now be considered more in detail. Considerable amounts of organic reserve food materials are stored in all seeds, either in the cotyledons or in the endosperm. Consequently, the first phases of germination can occur without light or mineral substances. During germination in darkness stored substances alone are utilized, the chemical nature and amount of which can be determined by exact analysis. Quite similar phenomena occur also in plants growing in light; but matters are complicated in this case by the fact that the process is accompanied by the assimilation of carbon dioxide and mineral constituents. This assimilation results in the formation of new substances, of external origin, which obscure the transformations occurring in the reserve materials. By studying germination in darkness and in distilled water we may eliminate the absorption of all materials except water and atmospheric oxygen, and may thus study the changes of reserve materials already within the plant.

It is generally observed that the dry weight of seedlings of various plants is considerably less than the dry weight of the ungerminated seeds. This is illustrated by the following analyses of 46 wheat seeds and of the same number of seedlings. The numbers represent grams.

	Total Dry Weight	Carbon	Hydrogen	Oxygen	Nitrogen	Total Ash
Seeds	1.665	0.758	0.095	0.718	0.057	0.038
Seedlings	0.722	0.293	0.043	0.282	0.057	0.038
Loss during germination	0.943	0.465	0.052	0.436	0.000	0.000

The chemical processes of germination are not identical in different kinds of seeds; they depend largely upon the chemical nature of the stored reserve materials. Seeds are grouped into three classes according to the nature of the reserve materials that predominate in them: starchy seeds, proteinaceous seeds and fatty seeds.

From the table given above it is evident that the loss of material during the germination of starchy seeds (such as those of the Gramineæ[1]) occurs through loss of carbon, oxygen and hydrogen. The amount of nitrogen and of ash constituents remains unchanged. The nature of the transformations occurring in the germination of maize is shown in the following table, which presents the results of analyses of 22 maize seeds and of as many seedlings. The numbers represent grams. It thus appears that most of the starch is decomposed by diastase, with the formation of glucose and cellulose.

During the germination of proteinaceous seeds also (such as those of the

[1] Boussingault, 1860–1891. [See note 5, p. 2.] Vol. 4.

Leguminosæ[1]), the decrease in dry weight is due to loss of carbon, hydrogen and oxygen. Protein decomposition occurs at the same time, with the formation of asparagin and amino acids. Glucose is formed from the non-nitrogenous

	Total Dry Weight	Starch and Dextrine	Glucose and Saccharose	Fats	Cellulose
Seeds	8.636	6.386	0.000	0.463	0.516
Seedlings	4.529	0.777	0.953	0.150	1.316
Gain or loss during germination	−4.107	−5.609	+0.953	−0.313	+0.800

substances, and the cellulose content increases. The table given below shows analyses of seeds and seedlings of *Lupinus luteus*, the quantities being percentages of the total dry weight of the seeds. Sulphates also appear, as a by-

	Total Dry Weight	Proteins	Asparagin	Other Nitrogenous Substances	Glucose	Cellulose
Seeds	100.00	45.07	0.00	11.6	0.00	3.24
Seedlings	81.70	11.06	18.22	23.97	2.10	6.47
Gain or loss during germination	−18.30	−34.01	+18.22	+12.31	+2.10	+3.23

product of protein decomposition in the germination of proteinaceous seeds; lupine seeds with an equivalent sulphuric acid content of 0.385 g. were germinated and showed a corresponding content of 0.610 g. when seven days old, and one of 1.323 g. when fifteen days old.

In the germination of fatty seeds (such as those of Helianthus, Cucurbita, Ricinus, etc.[2]) the loss in dry weight occurs almost entirely through loss of carbon and hydrogen, while the amount of oxygen actually increases through absorption. The stored fats decrease during this process and are replaced by starch, which shows how the absorption of oxygen is to be interpreted. Fats are much poorer in oxygen than starch, and the formation of starch from fats is therefore possible only with addition of oxygen. The hydrolysis of fat results in an increase in the fatty acid content of the seeds during germination. The following experiment may serve as an illustration of this. Twenty grams of poppy seed contained 8.915 g. of fat and 0.975 g. of free fatty acids. After germination for four days 3.77 g. of free fatty acids were present, and only 3.90 g. of fat. Glycerine, however, was not found in the seedlings.

The following table illustrates the changes that occur during the germination of sunflower seeds. The numbers represent percentages, on the basis of the original total dry weight of the seeds.[3]

[1] **Schulze, E.,** and **Umlauft, W.** Untersuchungen über einige chemische Vorgänge bei der Keimung der gelben Lupine. Landw. Jahrb. **5**: 821–858. 1876.

[2] **Laskovsky,** Die Keimung der Kürbissamen in chemischer Bezielhung. Moscow, 1874.*

[3] **Frankfurt,** 1894. [See note 5, p. 186.]

	Seeds	Seedlings	Gain or Loss During Germination
Total dry weight	100.00	88.98	−11.12
Simple proteins	24.06	13.34	−10.72
Nuclein and plastin	0.96	4.05	+ 3.09
Asparagin and glutamin	0.00	3.60	+ 3.60
Lecithin	0.44	0.71	+ 0.27
Fats	55.32	21.82	−33.50
Sugars	3.78	13.12	+ 9.34
Soluble organic acids	0.56	2.16	+ 1.60
Cellulose	2.54	10.25	+ 7.71
Hemicelluloses	0.00	3.41	+ 3.41

The main facts regarding germination may of course be most readily demonstrated from the study of seeds germinated in darkness. Germination in light is identical with that in darkness except for the additional assimilation of carbon and mineral constituents.[1]

Summary

1. **The Cell as Physiological Unit.**—The life activities of a plant are the summed activities of its cells. Cell activities are due to protoplasm, influenced by the surroundings. In all but the very simplest forms, the protoplasm of each cell consists of the nucleus and the cytoplasm, and both parts are necessary for the life of the cell. Plastids are special parts of the protoplasm. Chemically, the protoplasm consists largely of water and proteins. Over 90 per cent. of the weight of active protoplasm is water. Among the non-aqueous substances in protoplasm the proteins predominate, forty per cent. of the dry weight of slime-mould plasmodium (protoplasm mainly) being proteins.

2. **Proteins.**—The proteins are chemically the most complex substances in the plant. They are very plentiful in resting tissue (such as seeds), less plentiful in active tissue, and least plentiful in mature tissue that has nearly ceased its activities. Full-grown leaves that are still active photosynthetically contain much protein in their chloroplasts. There are several chemical tests generally used for identifying proteins.

The proteins of plants are considered as belonging to two groups, the simple proteins (such as the albumin of aleurone in seeds) and the compound proteins (which are essential in the protoplasm itself). The simple proteins contain carbon, hydrogen, oxygen, nitrogen, and often sulphur. They are combinations or complexes of much simpler nitrogenous organic compounds, the amino acids. About seventeen different amino acids enter into the simple proteins of plants (and animals also). The simplest of these acids is *glycocoll* (alpha-amino-acetic acid, CH_2NH_2COOH). As examples of the most complicated amino acids may be mentioned *cystin* (alpha-diamino-beta-dithio-dilactic acid) and *triptophan* (beta-indol-alpha-amino-propionic acid). The constituent amino acid molecules are joined into groups called polypeptides, and polypeptide groups are united to form the simple protein molecule. The simple proteins, of which there are a large number, act as foods and are not considered here

[1] For a treatise on seed germination see: **Detmer, Wilhelm,** Vergleichende Physiologie des Keimungsprocesses der Samen. Jena, 1880.

as really constituents of the protoplasm, though they occur in the cytoplasm of cells. They mainly occur as protein grains or dissolved in the cell sap (which is the more liquid material lying within the cytoplasm of ordinary plant cells).

The nucleo-proteins furnish examples of the complex proteins. They are combinations of simple proteins with nucleins, and nucleins are combinations of the more complex simple proteins with nucleic acids. The latter are rich in phosphorus. The simplest nucleic acid (derived from yeast) has the formula, $C_{40}H_{59}N_{14}O_{22}—2P_2O_5$. These acids give phosphoric acid, on being decomposed.

3. **Enzymes.**—Enzymes are catalyzers that occur in plant cells. They are organic in their nature and act, like other catalyzers, to accelerate (or retard) chemical changes or to alter the equilibrium point at which a chemical change ceases. In their presence many chemical reactions go on that would not go on appreciably without their aid, or would soon come to equilibrium and cease. The rate of change depends on the amount of enzyme present, as well as upon the temperature and the other substances in the medium. The enzyme is not used up in the process that it promotes. The chemical nature of enzymes is not yet known; they are known by their effects.

Diastase is a widely distributed plant enzyme. It causes the transformation of starch into glucose, with the consumption of water. It may be obtained from germinating seeds, such as those of barley (malt), from leaves, etc. Diastase, as here defined, consists of a mixture of two enzymes, *amylase* and *maltase*. Amylase transforms starch to maltose, and maltase forms dextro-glucose (dextrose) out of maltose.—*Saccharase* (or invertase) is another common enzyme in plants. It converts saccharose (cane sugar) into dextro-glucose (dextrose) and dextro-fructose (levulose), with the consumption of water.—Proteins are transformed and rendered soluble in water by the action of *proteolytic enzymes*, which decompose proteins, with consumption of water, into simpler compounds.—Fats are decomposed into glycerine and fatty acids, with consumption of water, by *lipases*.

Besides these, and other, hydrolytic enzymes (operating with consumption of water), *oxidases* are present in plant cells. These cause the oxidation of organic substances, with molecular oxygen.—Another non-hydrolytic enzyme is *zymase*, which promotes the formation of alcohol and carbon dioxide from glucose. It occurs plentifully in active yeast cells.

Living protoplasm is supposed to contain specific enzymes that promote the various chemical changes of vital activity, not only decompositions but syntheses. Hill showed that maltase acts in either direction, to form glucose from maltose (with addition of water) or to form maltose from glucose (with production of water), according to the concentrations of maltose and glucose in the mixture.

Tissues may be killed without destroying all of their enzymes, as follows from the fact that many enzymes may be extracted from tissues that have been killed in the process of extraction. Enzymes may remain active in dead tissues for a considerable time, but enzyme activity in dead cells is not automatically regulated and coördinated as it is in living cells. The enzymes are very important agents in vital processes, and perhaps catalysis furnishes a key to vital processes in general, but the nice coördination of all the various catalytic actions that is the main characteristic of living protoplasm remains still to be understood.

4. **Protein Decomposition in Plants.**—Proteins are continually broken down and re-formed in living tissues, apparently by the action of enzymes. In germinating seeds, the decomposition of simple proteins gives amino acids such as tyrosin (beta-para-hydroxyphenyl-alpha-amino-propionic acid) and leucin (alpha-amino-isobutyl-acetic

acid). Asparagin ($NH_2COCH_2CHNH_2$) appears to be formed from these in most plants, in the presence of oxygen, so that the amino acids do not accumulate except when oxygen is absent. In the presence of oxygen asparagin appears as the main nitrogenous waste of plants (somewhat as urea (NH_2CONH_2) does in animals). But this waste is not given off to the exterior of the plant; in green leaves, in sunlight (apparently because of a plentiful supply of sugars), asparagin is combined with carbohydrates, forming simple proteins, and thus returns to the metabolic system. Only in very young seedlings (in the presence of oxygen) does asparagin accumulate considerably, since it is used up in protein synthesis about as rapidly as it is formed; but it becomes clearly evident in older plants kept for a time in darkness, where protein formation is stopped because of lack of carbohydrates.—The decomposition of proteins, and the products formed, are influenced by the chemical nature of the substances supplied to the living cells. The mould Aspergillus forms oxalic acid when grown in acid media, but when there is an excess of calcium carbonate it forms tyrosin and leucin instead of oxalic acid.

The compound proteins are also broken down in active tissues, and their decomposition products appear, especially in darkness and when the plants are starved. Apparently the simple proteins are attacked first (and as long as the supply lasts), and the complex proteins are considerably decomposed only when the supply of simple proteins is about exhausted. Compound proteins appear in many instances to be formed at the expense of simple proteins.

5. Nitrogenous Products of Protein Decomposition.—As has been said, asparagin appears as the most important decomposition protuct of simple proteins. In some plants its place is taken by a similar substance, glutamin. Tyrosin, leucin, and some other amino-acids are also formed in plants, from simple proteins. The purin bases (xanthin, hypoxanthin, adenin, guanin), as well as their derivatives (such as caffein —the main alkaloid of coffee and tea, and theobromin—the main alkaloid of chocolate) result from the decomposition of the nucleo-proteins.

6. Protein Synthesis in Plants.—The primary synthesis of simple proteins in ordinary green plants appears to occur in the leaves, where carbohydrates (formed in the chlorophyll-bearing tissues by photosynthesis) are combined with the nitrogen of nitrates (which reach the leaves through the xylem vessels, from the absorbing regions of the roots). Nitrates are usually found in leaves only in very small amounts, and it appears that they are ordinarily used up as rapidly as they arrive. In prolonged darkness, however, the supply of carbohydrates is stopped, and nitrates accumulate in leaves to considerable amounts. Also, nitrates have been found to accumulate in the chlorotic parts of white-green variegated leaves, even in light. In these parts no carbohydrates are formed. Light is apparently not directly necessary for the synthesis of simple proteins from carbohydrates and nitrates, but it is of course necessary for the photosynthesis of carbohydrates, and it is thus indirectly necessary for these protein syntheses.

Hydrocyanic acid (HCN) has been supposed to be an intermediate product in simple protein synthesis from carbohydrates and nitrates. Many leaves ordinarily contain this acid (combined with sugars) in detectable quantities. It disappears, however, after prolonged darkness (being probably used up in protein formation), but it reappears when the leaves are supplied with nitrate and sugar in darkness or when they are supplied with nitrate in light.

Decomposition of simple proteins occurs in germinating seeds, in darkness, as has been said, while the later growth of the plantlets (in light and with photosynthesis

going on) shows protein synthesis. Light is here, again, apparently necessary only for the formation of the necessary carbohydrates.

As to the nucleins (of compound proteins), active growth is generally accompanied by nuclein synthesis. The nucleo-proteins increase during the first stages of seed germination, doubtless being formed at the expense of the simple proteins, which decrease during these stages. Compound proteins are formed in leaves when sugars are plentiful, and the process seems to be more rapid in light than in darkness, as though light exerted a direct influence in this case.

7 [8]. **Lipoids and Phosphatides.**—Lipoids are substances that can be dissolved out of plant or animal cells by treatment with ether or similar solvents. Here belong not only fats and fatty acids, but also phosphatides. The latter may be defined as lipoids containing phosphorus; they are very active chemically and are present in all protoplasm. Lipoids apparently form labile compounds with the proteins, on the one hand and with iron, calcium, etc., on the other hand.

8 [9]. **Carbohydrates.**—The carbohydrates of plants are either water-soluble (glucose, saccharose, inulin, etc.) or insoluble in water (starches, celluloses, etc.). Starch and cellulose appear to be formed in plant cells by a sort of decomposition of proteins. Protoplasm is necessary for their formation, and they form as solid masses in leucoplasts (starch grains) or at the protoplasmic periphery (cell walls). There is evidence that their formation is accompanied by the production of nitrogenous substances such as asparagin. (These nitrogenous products appear to be then combined with sugar, forming more proteins, so that cellulose and starch are said to be formed by a sort of condensation of sugar, although proteins seem to be involved in the process.) Cellulose forms the main mechanical support of plant tissues, but the thickened cell walls of some forms (as the endosperm of date seeds) are subsequently dissolved, by the action of the enzyme cytase, and furnish sugar that is used in later growth. Ordinary cell walls contain *hemicelluloses*(which can be extracted with hot 1 per cent. solution of hydrochloric or sulphuric acid) and *true celluloses* (which cannot be extracted in that way). The cell walls of many fungi (ordinary mushrooms) are composed of *fungus cellulose*, which contains considerable nitrogen and is similar to the *chitin* of the external skeleton of insects, crabs, etc.

Grape sugar (dextrose or dextro-glucose) is a glucose sugar very generally present in plant cells. It is one of the simplest of the carbohydrates, being represented by the formula $C_6H_{12}O_6$. As has been said, it is freely soluble in water and always occurs in aqueous solution in the tissues. Saccharose (cane sugar) is a more complex water-soluble carbohydrate, represented by the formula $C_{12}H_{22}O_{11}$. It is very common in plants (in fruits, roots, stems, etc., in large amounts) and forms dextrose (grape sugar) and levulose (fruit sugar, fructose) upon partial decomposition, as by the enzyme invertase.

9 [10]. **Glucosides.**—Glucosides are chemical combinations of glucose, or other sugars, with various organic substances, and they decompose into these constituents, with the consumption of water, when acted upon by glucoside-splitting enzymes or by acids. For example, *amygdalin* is a glucoside occurring in leaves, seeds, etc., of almond, peach, etc. Under the influence of the enzyme emulsin, amygdalin takes up water and produces glucose sugar, benzaldehyde, and hydrocyanic acid.

10 [11]. **Organic Acids.**—Organic acids and their calcium salts, etc., occur commonly in plant cells. They are apparently formed by the incomplete oxidation of sugars. In some plants and in some tissues (leaves of Oxalis, petioles of rhubarb, fruit of lemon) they accumulate in large quantities. Various conditions influence

their formation; sugar must be plentifully supplied and, in some cases at least (as in some roots), oxygen must not be too plentiful.

11 [7]. **Alkaloids, Toxins, Antitoxins.**—A very large number of different kinds of substances, some of which are very poisonous, are formed in plants. Here may be mentioned solanin, an alkaloid that is formed in the potato, especially in wounded or actively growing regions. Bacteria form poisonous substances called toxins, which diffuse out of the cells into the surroundings. Some bacteria are parasitic and develop in the bodies of living higher plants and animals, these being the *pathogenic* forms. Pathogenic, as well as other, bacteria are generally grouped as *aerobic* or *anaerobic*, accordingly as they require free oxygen or are poisoned by it. Some forms (the *facultative* anaerobes) can live without free oxygen but are not injured by its presence.

The tetanus bacillus is an anaerobic form. Its development in the human body is confined to the neighborhood of the infected wound, but the very active toxin that it produces spreads throughout the body of the victim, causing death by lock-jaw. The anthrax bacillus, which causes splenic fever of cattle, etc., is aerobic and can be grown in bouillon. It was through Pasteur's studies of this organism that he discovered the bacterial nature of infectious diseases. He also laid the foundations of serum therapy, vaccination for immunization, etc., from experiments with anthrax bacillus. The peculiar toxin emanating from the cells of the parasitic bacillus stimulates the cells of the diseased animal to give off, into the blood-stream, a specific antitoxin. This tends to counteract the poisonous influence of the toxin, and if sufficient antitoxin can be formed soon enough (or is supplied from the blood serum of another animal that has been immunized by previous vaccination with a weakened strain of the parasite), the disease is cured. Diphtheria and tetanus are treated in this way. The solution obtained by filtering (through a Chamberland filter) a bouillon culture of the diphtheria bacillus is very poisonous because of the toxin that is present.

12. **Water.**—Physiological processes cannot go on without a plentiful supply of water; the chemistry of life is almost entirely that of aqueous solution. Active plant tissues contain 80 or 90 per cent. (by weight) of water. Resting cells (as in seeds) become very dry, but they must be freely supplied with water before they become active. The water-soluble substances of the organism are largely dissolved in the water of active cells, and many insoluble materials swell in water until the colloid dispersion formed has many of the properties of a true solution. Electrolytes become ionized (and consequently more active chemically) when in aqueous solution. Furthermore, water itself enters into numerous chemical reactions in living cells; for example, the photosynthesis of carbohydrates, and the hydrolytic decompositions of carbohydrates, proteins, etc., by enzymes. Practically all of the hydrogen of the plant is derived from water. Some water is produced in living cells, through respiration, through the formation of the complex carbohydrates, proteins, etc., from simpler substances, but by far the greater part of the water in a plant has entered as such, from the soil. Much water escapes from ordinary plants by transpiration, some by guttation, glandular secretion, etc.

13. **Germination of Seeds.**—The resting seed contains all the substances needed for a considerable amount of growth, excepting water and oxygen; the latter substances are absordeb as germination starts and proceeds. The earlier stages of germination, until the photosynthesis of carbohydrates becomes pronounced, result in the using up of the non-aqueous materials of the seed; thus the dry weight of a young seedling is smaller than that of the ungerminated seed.

The chemical processes of germination are different in different kinds of seeds. Seeds may be grouped into three classes according to the substances that predominate in them: starchy seeds, proteinaceous seeds, and fatty seeds. In starchy seeds (such as the cereal grains) carbon, hydrogen, and oxygen are lost during germination. Germinating proteinaceous seeds (such as those of the legumes) also give of carbon, hydrogen, and oxygen, but they are specially characterized by the production of asparagin, amino acids, and some sulphates. Fatty seeds (such as those of sunflower) do not lose oxygen during germination; the oxygen content actually increases. The original supply of fats is depleted and carbohydrates appear, these being apparently formed by the oxidation of the fats. Free fatty acids increase in amount.

CHAPTER VIII

FERMENTATION AND RESPIRATION

§1. **General Discussion.**—Plants grow, and in growing they produce various metabolic changes and movements of materials. It thus comes about that work of various kinds is performed in living plants, and this necessitates the consumption of energy. The organic substances produced by green plants in sunlight are sources of energy to the plant, just as wood, gasoline or coal may act as the source of energy for the operation of a manufactory, the energy necessary for the running of the machinery being supplied by the combustion of such materials. The processes of living plants in which organic reserve substances are oxidized by oxygen are quite analogous to combustion, and this vital oxidation is known as respiration.

The material changes that constitute respiration may be considered as consisting typically in the absorption of oxygen and the formation of carbon dioxide and water, the latter remaining in the plant body.[a] The general process may be represented by the equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\,O_2} = \underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{6\,H_2O}.$$

It is thus clear that these material changes of respiration proceed in a direction opposite to that of the photosynthetic process. Respiration results in the decomposition of material by oxidation. It is really a kind of slow combustion and, like other kinds of combustion, it is accompanied by the liberation of energy. This liberated energy is used in other processes that go on within the plant, or some of it may escape to the surroundings. The loss of material from seeds germinating in darkness is due to this process. A part of the reserve material of the seed is oxidized, and the energy thus liberated is largely used in the construction of the young plant out of the remaining material.

Normal respiration does not occur everywhere in nature. Atmospheric oxygen fails to penetrate into many places where organisms may develop, as in the case of stagnant water and especially in flooded soils. Hoppe-Seyler[1] has suggested some simple criteria for judging whether or not a soil contains oxygen. Moor soil that is nearly free from oxygen is peculiarly colored. Also, the formation of methane, hydrogen sulphide, ferrous carbonate and

[1] **Hoppe-Seyler,** Über die Einwirkung des Sauerstoffs auf Gärungen. Strassburg. 1881. P. 26.*

[a] There seems to be no reason for supposing that respiration water is less apt to pass out of the plant body than is water from any other source. This water must simply become a part of the general water mass of the organism and the water lost by transpiration and excretion, as well as that chemically fixed in photosynthesis and hydrolysis, is supplied from this general mass. In this connection it may be recalled that the ordinary plant loses very much more water than any other substance, during its growth.—*Ed.*

ferrous sulphate takes place in the absence of oxygen. On the other hand, the presence of ferric hydrate in a soil indicates an adequate supply of oxygen for plant growth.

Various simple plant forms always abound in soil and water that lack oxygen. Since the absorption of free oxygen is impossible under such conditions, the energy requirements of these organisms must be supplied by processes other than those of simple oxidation. As a matter of fact, such processes—which are those of fermentation, in general—do occur in organisms that exist without free oxygen.

It is well known that energy is liberated by the decomposition of many organic substances in other ways, as well as by oxidation processes. Berthelot[1] showed that formic acid is decomposed by platinum black, into carbon dioxide and hydrogen, with liberation of heat, the reaction being represented by the equation:

$$\underset{\text{Formic acid}}{HCOOH} = \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Hydrogen}}{H_2}.$$

On the basis of this observation he concluded that heat production may occur in living organisms without any relation to oxidation processes.

Oxidation,[b] with liberation of heat, may occur also *in the absence of molecular oxygen*, this element being derived from water. Wieland[2] showed that, in the presence of palladium-black, aldehydes are oxidized by water, to form the corresponding acids. Hydrogen is liberated and absorbed by the palladium black. The reaction is represented as follows:

$$\underset{\text{Aldehyde}}{R\text{-}COH} + \underset{\text{Water}}{H_2O} = \underset{\text{Acid}}{R\text{-}COOH} + \underset{\text{Hydrogen}}{H_2}.$$

Loew[3] showed that much hydrogen is freed from an alkaline solution of formaldehyde in the presence of cuprous oxide, formic acid being formed. This reaction explains the formation of fatty acids, with evolution of hydrogen, by anaerobic bacteria. These bacteria effect oxidation in the absence of molecular oxygen, deriving this element from water. Favorskii[4] cites a series of oxidations of organic compounds at the expense of water.

Finally, oxidation in the absence of free oxygen may occur as the result of the removal of hydrogen from a molecule, so as to form carbon dioxide. Thus, by the action of sunlight on a mixture of formic acid and quinone, Ciamician

[1] **Berthelot, Marcellin,** Sur le synthèse de l'acide formique. Compt. rend. Paris, 59: 616–618. 1864. **Idem,** Sur l'acide formique. *Ibid.* 59: 817–819. 1864. **Idem,** Sur la décomposition de l'acide formique. *Ibid.* 59: 861–865, 901–904. 1864.

[2] **Wieland, Heinrich,** Studien über den Mechanismus der Oxydationsvorgänge. Ber. Deutsch. Chem. Ges. 45^{II}: 2606–2615. 1912.

[3] **Loew, O.,** Ueber einige katalytische Wirkungen. Ber. Deutsch. Chem. Ges. 20^{I}: 144–145. 1887.

[4] **Favorskii, A. E.,** Ueber Isomerisationserscheinungen in den Reihen der Carbonylverbindungen gechlorter Alcohole und haloidsubstituirter Oxyde der Aethylenekohlenwasserstoffe. (Original in Russian, St. Petersburg, 1895.) Rev. in Jour. prakt. Chem. 51: 533–563. 1895. Rev. also in Bull. Soc. Chim. Paris 14: 1188–1206. 1895.

[b] This and the next following paragraph are introduced from the 7th Russian edition.—*Ed.*

and Silber[1] obtained hydroquinone and carbon dioxide, according to the following equation:

$$\underset{\text{Formic acid}}{HCO_2H} + \underset{\text{Quinone}}{C_6H_4O_2} = \underset{\text{Hydroquinone}}{C_6H_6O_2} + \underset{\text{Carbon dioxide}}{CO_2}.$$

Bredig and Sommer[2] also obtained carbon dioxide by the action of methylene blue on formic acid in the presence of a catalyzer, the reaction being:

$$(C_{16}H_{18}N_3S)_2SO_4 + HCO_2H = (C_{16}H_{20}N_3S)_2SO_4 + CO_2.$$

Fermentation processes are really processes of decomposition accompanied by the liberation of heat, and they may take the place of respiration when free oxygen is not absorbed. Pasteur regarded fermentation as "life without oxygen." Economically these decompositions are less efficient for the organism than are oxidations, for more energy is always liberated in the latter. It is obvious, for example, that the oxidation of formic acid must produce a greater amount of heat than does the simple decomposition of this substance into carbon dioxide and hydrogen, since the heat of combustion of hydrogen does not appear in the latter case. An analogous result is reached by comparing the equation representing oxygen respiration with that for alcoholic fermentation, from the thermo-chemical point of view.

$$\text{Respiration: } C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + 6\,H_2O.$$
$$\text{Fermentation: } C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2.$$

In the first case the total heat of combustion of the glucose is liberated, which amounts to 709 kg.-cal. per gram-molecule (180 g.). The amount of heat liberated in the second case must be less than in the first, because one of the end products of fermentation is ethyl alcohol, which is easily oxidized. This alcohol gives a heat of total combustion of 326 kg.-cal. per gram-molecule, and, since there are two molecules of alcohol produced from each molecule of glucose, we must subtract 2×326 from 709, thus obtaining 57 kg.-cal. as the amount of heat set free by the fermentation of a gram-molecule of glucose according to the second equation given above. It follows that more than twelve times as much glucose must be decomposed in fermentation as is oxidized in respiration, to give equal amounts of free heat. The difference between the two processes is practically even more pronounced than is thus indicated. All kinds of fermentation require relatively very large amounts of material, as compared with the corresponding complete oxidations.

Fermentation consists in the decomposition of organic compounds without the agency of atmospheric oxygen, while respiration is essentially an oxidation process. The question now arises whether there may be a relation-

[1] Ciamician, G., and **Silber, P.**, Chemische Lichtwirkungen. (I Mitteilung.) Ber. Deutsch. Chem. Ges. 34^{II}: 1530–1543. 1901.

[2] **Bredig, G.**, and **Sommer, Fritz**, Anorganische Fermente. V. Die Schardingersche Reaktion und ähnliche enzymartige Katalysen. I. Die Schardingersche Reaktion mit anorganischen Fermenten. [Reduktion von Methylenblau mit Formaldehyd durch Metallkatalyse.] Zeitsch. physik. Chem. **70**: 34–65. 1910.

ship between the two, as they occur in organisms; this question was first answered in the affirmative by Pflüger,[1] whose conclusions in this regard are now generally accepted. In living animals and plants various kinds of organic decompositions are always going on, under the influence of specific intracellular enzymes. In some cases, as in the microörganisms that produce various kinds of fermentation, the entire energy requirement is supplied in this way. Oxidation of the decomposition products thus formed may fail to occur here, either because the organisms in question live in the absence of oxygen or because they lack the necessary oxidation enzymes. It may also occur that the fermentation products diffuse out of the cells before oxidation can occur, especially in the case of organisms that develop in a liquid medium. Most plants, however, absorb oxygen by means of their oxidizing enzymes, thus allowing the complete oxidation (to water and carbon dioxide) of the decomposition products that arise from the breaking down of complex nutrient materials. This constitutes aerobic or normal respiration. If ordinary plants are deprived of free oxygen, then their respiratory processes become restricted to those of fermentation, which is thus seen to be a fundamental process characteristic of all plants.

§2. **Alcoholic Fermentation.**[2]—Alcoholic fermentation consists essentially in the splitting of various sugars into ethyl alcohol and carbon dioxide through the specific activities of organisms such as the Saccharomycetes; negligible amounts of succinic acid and glycerine are also formed. This kind of fermentation occurs especially in the presence of yeast fungi. At first thought, it may appear that the fermentation of grape juice is an exception to this statement, since yeast is not added to the juice, but Pasteur showed that yeast fungi are also effective here. Microscopic examination demonstrates the presence of various kinds of yeasts upon the outer surface of the fruit of the grape, and when the berries are pressed these pass into the juice, where they multiply and give rise to alcoholic fermentation. Yeast cells are not numerous on uninjured grapes, but berries that have been perforated by wasps often exhibit large colonies of well-nourished, budding cells. The yeasts find here a very favorable substratum for growth and reproduction, and the cells are carried from one bunch to another by the wasps. All of these insects are found to be carriers of yeast cells during the grape season, as may be shown either by direct microscopical examination of the wasps or by placing them in sterilized beer-wort and noting the subsequent fermentation that is set up. Wortmann performed many experiments of this kind, always with the same result; after the introduc-

[1] **Pflüger, E., F. W.,** Beiträge zur Lehre von der Respiration. I. Ueber die physiologische Verbrennung in den lebendigen Organismen. Pflüger's Arch. Physiol. 10: 251–367, 641–644. 1875. **Pfeffer, W.,** Das Wesen und die Bedeutung der Athmung in der Pflanze. Landw. Jahrb. 7: 805–834. 1878. **Wortmann, Julius,** Ueber die Beziehungen der intramolecularen zur normalen Athmung der Pflanzen. Arbeit. Bot. Inst. Würzburg. 2: 500–520. 1882.

[2] **Pasteur, L.,** Etudes sur la bière. Paris, 1876.* **Mortiz,** and **Morris,** 1891. [See note 3, p. 164.] **Lafar, Franz,** Technische Mykologie. Ein Handbuch der Gärungsphysiologie für technische Chemiker, nahrungsmittelchemiker usw. Jena, 1897–1907. **Idem,** Technical Mycology; the utilization of microörganisms in the arts and manufactures. A practical handbook, etc. Translated by Charles T. C. Salter. (2 vols. in 3.) London, 1903–1910. **Buchner, Buchner** and **Hahn,** 1903. [See note 2, p. 167.] **Duclaux,** 1899–1900. [See note 2, p. 163.] **Hansen,** 1896. [See note 1, p. 44.] **Oppenheimer,** 1909. [See note 2, p. 163.] **Wahl** and **Henius,** American handy book of brewing, malting and auxiliary trades. [Chicago, 1902.]

tion of the wasp the medium soon began to ferment. The yeast cells pass the winter in the soil and find their way to the young fruits the following season.

Fermentation results in an increase in the dry weight of the yeast. If a fermentable liquid is inoculated with a slight amount of yeast, the cells rapidly increase by budding, and if enough of the liquid has been used a considerable amount of dry substance is finally obtained. Fermentation is thus a physiological process connected with the growth and reproduction of the yeast cells.

Glucose and other varieties of sugar are suitable material for fermentation. *Saccharomyces cerevisiæ I*, *S. pastorianus I*, *II* and *III*, and *S. ellipsoideus I* and *II*, all contain the enzyme invertase, which hydrolyzes cane sugar to form fructose and glucose, the latter being subject to fermentation. Maltose is fermented in the same way, but lactose is not affected. *Saccharomyces marxianus*, *S. ludwigii* and *S. exiguus* attack only glucose and saccharose, without affecting lactose or maltose; *S. apiculatus* ferments only glucose, but *S. kiphyr* and *S. lactis* are able to hydrolyze lactose.

Sugar solution alone fails to produce an abundant growth of yeast; nitrogen and mineral substances are necessary for these cells just as in the case of other plants. These other substances are plentiful in grape juice and beer-wort, but must of course be included in artificial nutrient media if yeasts are to be cultivated therein. Among the ash-constituents of yeast, phosphates play a conspicuous rôle.

The researches by Harden and Young[1] indicate that alcoholic fermentation proceeds by two stages, as follows:

$$\text{1.}\quad \underset{\text{Glucose}}{2C_6H_{12}O_6} + \underset{\text{Phosphate}}{2M''HPO_4} = \underset{\text{Carbon dioxide}}{2CO_2} + \underset{\text{Alcohol}}{2C_2H_5OH} + \underset{\text{Water}}{2H_2O} + \underset{\text{Hexose phosphate}}{C_6H_{10}O_4(M''PO_4)_2}$$

$$\text{2.}\quad \underset{\text{Hexose phosphate}}{C_6H_{10}O_6(M''PO_4)_2} + \underset{\text{Water}}{2H_2O} = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Phosphate}}{2M''HPO_4}$$

Hexose-phosphate is thus formed and again decomposed during the process, and it is for this reason that the addition of soluble phosphates accelerates fermentation. The phosphate may therefore be considered as a co-enzyme of zymase. Harden and Young showed that after the filtration of yeast through a gelatine filter neither the filtrate nor the precipitate is capable of producing alcoholic fermentation, but fermentation does occur if the filtrate and precipitate are again brought together. The necessary phosphates occur in the filtrate in this experiment.

Yeast cells may also develop in a medium *without nutrient material*, under otherwise suitable conditions, and they still produce carbon dioxide and alcohol. This is the so-called auto-fermentation of yeast, which results in a decrease rather than in an increase of dry substance. Here the carbon dioxide and alcohol are formed at the expense of the yeast material itself. A similar phe-

[1] **Harden, Arthur,** and **Young, William J.,** The alcoholic ferment of yeast-juice. Proc. Roy. Soc. London 77: 405–420. 1906. **Idem,** same title. *Ibid.* 78: 369–375. 1906. **Idem,** same title. *Ibid.* 80: 299–311. 1908. **Idem,** The function of phosphates in alcoholic fermentation. Centralbl. Bakt., *II*, 26: 178–184. 1910.

nomenon appears in the germination of seeds in darkness, where the loss in dry weight is due to respiration in the absence of the photosynthetic process.

Great interest is attached to the question of the rôle of oxygen in alcoholic fermentation. Pasteur devised the apparatus shown in Fig. 89 for experiments upon the development of yeast in the complete absence of oxygen. A fermentable liquid is placed in the flask *A*, which has two glass necks (*a* and *b*) with narrow openings. One of these is provided with a glass stop-cock and a glass funnel while the other bends downward into a dish (*c*) filled with some of the same liquid as is in the flask. Both masses of liquid are brought to boiling, to expel air from the liquid. After cooling, the liquid in the dish is replaced with mercury. Resting yeast cells are then introduced into the glass funnel and admitted into the flask through the stop-cock. It was found that such resting yeast cells (called "old" cells by Pasteur) produce no fermentation when air is entirely lacking. In another series of experiments a small amount of the fermentable liquid was introduced into the funnel, inoculated with yeast, and fermentation was allowed to take place. A little of the fermenting liquid, containing a very few of the young, budding cells was then allowed to pass from the funnel into the flask, the cock being immediately reclosed. Vigorous fermentation occurred in the flask, more than a gram of dry substance being obtained from the very slight amount of yeast that was introduced. It is clear, therefore, that oxygen is essential to the development of resting yeast cells, while young cells can develop when oxygen is entirely lacking, if nutrient materials are present.

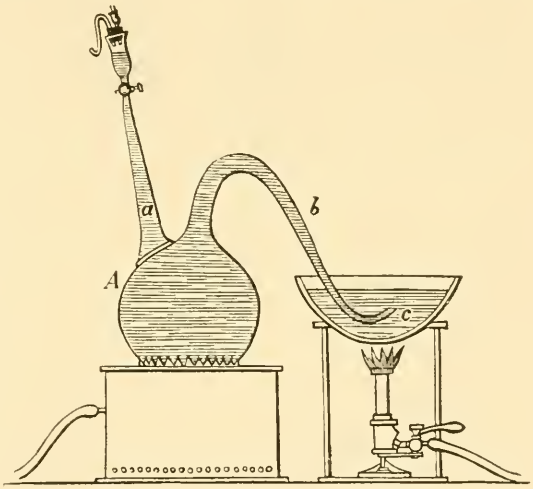


FIG. 89.—Apparatus for showing fermentation in the absence of oxygen.

In connection with the relation of oxygen to fermentation, it is of great importance to discover whether normal respiration occurs in yeast abundantly supplied with oxygen. Ivanovskii,[1] who took up this question, grew a pure culture of yeast upon a sterilized porous clay plate half immersed in sterilized nutrient solution, the whole being in an air chamber formed by a bell-jar. The yeast was thus abundantly supplied with oxygen, and the nutrient solution reached the cells only through the capillary passages of the clay plate. After three days a gas analysis showed that the ratio between the amount of carbon dioxide eliminated and the amount of oxygen absorbed, $\left(\frac{CO_2}{O_2}\right)$, was

[1] **Ivanovskii, D.,** On the influence of oxygen on alcoholic fermentation. [Russian.] Works of the Botanical Laboratory, Acad. Sci. St. Petersburg. No. 4. In Zapiski Acad. Sci. St. Petersburg 73^2. 28 p. 1894. [Pagination of parts in vol. is separate.]

equal to $\frac{2.0}{0.2}$, or 10. It thus appears that but very little oxygen is absorbed, even with an abundance of this gas, while much carbon dioxide is produced; oxygen respiration is here very weak but the decomposition of sugar into alcohol and carbon dioxide is very pronounced. Another series of experiments by Ivanovskii gave concordant results. Equal amounts of nutrient solution were placed in two vessels, the space above the liquid being filled with air in one case and with nitrogen in the other, and equal quantities of yeast were added to the vessels. At the end of the experiment the rate of sugar fermentation, per gram of dry yeast, per day, was determined. In one test, for example, where the yeast introduced into each vessel had a dry weight of 0.16 g., this weight increased to 0.516 g. in the presence of air and to 0.497 g. in its absence. With air, 6.009 g. of sugar was decomposed in twenty-four hours and without air 5.804 g. Thus, the amount of sugar decomposed in twenty-four hours per gram of dry yeast was 8.9 g. in both cases. A marked difference between the two cultures is to be noted, however, in regard to their reproduction; with access of air the yeast multiplied considerably faster than in the absence of oxygen. With a long exposure to oxygen-free air, growth ceases entirely, but the cells still remain alive and capable of decomposing sugar. Reproduction continues indefinitely when the supply of oxygen is not cut off.

The researches of Gromow and Grigoriew [1] show that zymin (acetone-treated yeast, see page 174) produces carbon dioxide at the same rate in a stream of air as in a stream of hydrogen, and these results were substantiated by Buchner and Antoni.[2]

Palladin[3] showed that oxidation enzymes are present in yeast in but slight amount, and this explains the fact, which seems remarkable at first, that yeast produces alcoholic fermentation even with an abundant supply of oxygen. It is on account of the absence of these enzymes that yeast is unable to oxidize alcohol in the presence of air, but this organism usually develops in the absence of oxygen, where oxidation enzymes are not needed. Moreover, alcohol readily diffuses out of the cells and thus becomes inaccessible to the action of intracellular enzymes.

In the industries, it is well to carry out the fermentation process under conditions of good aeration, since the multiplication of the yeast is hastened by the presence of oxygen and the process is thus accelerated. Although each individual cell produces the same amount of alcohol in the absence as in the presence of air, the number of active cells is larger when oxygen is supplied. Oxygen thus exerts, indirectly, an accelerating influence upon fermentation.

The concentration of alcohol in the solution influences the rate of fermentation; with increasing alcoholic concentration an anesthesia of the yeast cells finally sets in, and the rate of sugar decomposition is diminished. If the alcoholic concentration reaches 16 per cent. fermentation ceases altogether.

[1] Gromow and Grigoriew, 1904. [See note 7, p. 174.]

[2] Buchner, Eduard, and Antoni, Wilhelm, Weitere Versuche über die Zellfreie Gärung. Zeitsch. physiol. Chem. 44: 206–228. 1905.

[3] Palladin, W., Ueber das Wesen der Pflanzenatmung. Biochem. Zeitsch. 18: 151–206. 1909.

Two kinds of fermentation are distinguished in the brewing industry: top-fermentation, which occurs at high temperatures, and bottom-fermentation, which occurs at lower ones, these two kinds of fermentation being produced by two different groups of yeast races. Experiments aiming to change bottom into top yeasts, or the reverse, have never been successful.

Pasteur called attention to the fact that the properties of beer depend upon the character of the yeast employed in its manufacture. Since bacteria cause a deterioration in beer, Pasteur suggested a method for yeast purification, by means of cultures with tartaric acid or phenol. Hansen proved, however (1883), that the most widespread and injurious "diseases" of beer are not caused by bacteria but are due to wild species of yeasts.[c] The same writer has also shown that treatment of yeast with tartaric acid fails to have any good effect and is positively harmful when wild species are present; such treatment weakens the cultivated yeast and the wild forms become ascendant in the culture. To obtain a perfect product pure cultures of yeast must be employed. Comparative studies have shown that different varieties of beer are produced from the same beer-wort by different forms of yeast. Thus, *Saccharomyces pastorianus I* produces a bitter taste and an umpleasant odor, while the use of *S. pastorianus III* or *S. ellipsoideus II* results in cloudy beers.

In a mixture of yeasts the wild forms may be identified by the time required for ascospore production at a temperature of 15°C., as is brought out by the following scheme.

Temperature	Wild Yeasts	Cultivated Yeasts, Fermentation Rate	
		Rapid	Slow
deg. C.			
15°	Ascospores after 72 hours	No ascospores after 72 hours	No ascospores after 72 hours
25°	Ascospores after 40 hours	Ascospores after 40 hours	Ascospores after 40 hours

If a drop of a yeast culture a day old is thinly spread on a sterilized plaster plate impregnated with beer-wort, and if the preparation is kept at a temperature of 15°C., no ascospores are found after seventy-two hours unless wild yeasts were present in the original culture; ascospore formation does not occur till later. If spores are found, on the other hand, then wild yeasts are present, and the amount of these may be estimated by the number of ascospores that have been formed. It is possible in this way to detect the presence of wild yeasts in mixtures where they comprise no more than one two-hundredth of the total amount of yeast present.

Another method of identifying yeasts is based on the forms of their "giant colonies,"[1] which are formed from cell masses.[d] A drop of a young yeast cul-

[1] **Lindner**, 1909. [See note 1, p. 44.]

[c] See **Hansen**, 1896. [See note 1, p. 44.]—*Ed.*

[d] This paragraph is omitted in the 7th Russian edition.—*Ed.*

ture in beer-wort is transferred to gelatine and the cells multiply and develop into giant colonies upon the gelatine surface. The form of colony is always constant for the same species and different forms of colonies are produced by different kinds of yeast. The drawings of Fig. 90 show how distinct are the giant colonies of various different yeasts.

It has been seen that alcoholic fermentation is a process involving the action of enzymes[e] (see page 204). Besides carbohydrates, such ketonic acids as pyrotartaric acid may also be decomposed in this way, as was shown by Neuberg[1] and his co-workers. Pyrotartaric acid is split into carbon dioxide and acetic

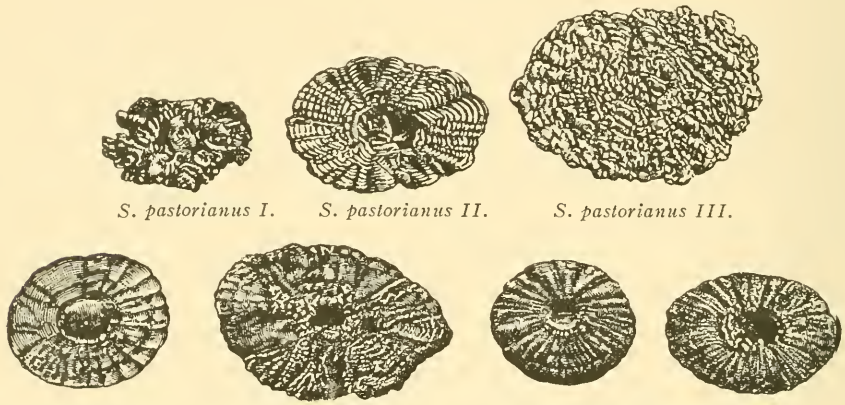


FIG. 90.—Giant colonies of different yeasts. (*After P. Lindner.*)

aldehyde, by a special enzyme, carboxylase, the reaction being represented by the following equation:

$$\underset{\text{Pyrotartaric acid}}{CH_3COCOOH} = \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Acetic aldehyde}}{CH_3COH}.$$

The acetic aldehyde thus formed is reduced to ethyl alcohol. Kostychev[2] reports that pyrotartaric acid is apparently one of the intermediate products in the breaking down of glucose. Zaliesskii[3] found the enzyme carboxylase in higher plants.

Reductase is plentiful in yeast, and this enzyme has been shown to play an

[1] **Neuberg, C.,** and **Karczag, L.,** Ueber zuckerfreie Hefegärungen. *IV.* Carboxylase, ein neues Enzym der Hefe. Biochem. Zeitsch. **36**: 68–75. 1911. **Idem,** same title. *V.* Zur Kenntnis der Carboxylase *Ibid.* **36**: 76–81. 1911. **Neuberg, Carl,** and **Kerb, J.,** Entsteht bei Zuckerfreien Hefegärungen Æthylalkohol? Zeitsch. Gärungsphysiol. **1**: 114–120. 1912.

[2] Kostytchew, S., Ueber Alkoholgärung. (I Mitteilung.) Ueber die Bildung von Acetaldehyd bei der alkoholischen Zuckergärung. Zeitsch. physiol. Chem. **79**: 130–145. 1912. **Kostyschew, S.,** and **Hubbenet, E.** (II Mitteilung.) Ueber Bildung von Aethylalkohol aus Acetaldyhyd durch lebende und getötete Hefe. *Ibid.* **79**: 359–374. 1912.

[3] Zaleski, W., Ueber die Verbreitung der Carboxylase in den Pflanzen. Ber. Deutsch. Bot. Ges. **31**: 349–353. 1913.

[e] This and the next following paragraph are not in the German edition and are translated from the 7th Russian edition.—*Ed.*

important rôle in alcoholic fermentation.[1] Palladin and Lvov[2] were able to retard the process of alcoholic fermentation by employing the respiration pigment of the white beet to remove the active hydrogen as it was formed. The production of alcohol was thus decreased, as well as that of carbon dioxide. They then employed methylene blue in place of the respiration pigment, and found that for each atom of hydrogen removed by the methylene blue there occurred a decrease of one molecule in the production of alcohol and of carbon dioxide. This dependence of alcoholic fermentation upon reduction processes may be represented by the following simplified scheme, in which M denotes methylene blue.

$$2\ C_6H_{12}O_6 + M = 2\ CO_2 + 2\ C_2H_5OH + C_6H_{10}O_6 + M\text{-}H_2.$$

The methylene blue is reduced to the leuco-compound. In this scheme no account is taken of Palladin's[3] opinion that alcoholic fermentation involves the chemical action of water, nor of Bach's idea that reduction also depends upon such action (see page 225).

The action of reductase consists in the removal of hydrogen from one substance (de-hydrogenation) and its transmission to another substance (hydrogenation).[f] The substance that gives up hydrogen is oxidized and is called the *reducer*, reductor or *reducing agent* ($R\text{-}H_2$). The other substance, said to be an *oxidizer* or *oxidizing agent*, which receives the hydrogen, is called the *acceptor* of hydrogen (A). The reaction is shown by the general equation,

$$R\text{-}H_2 + A = R + A\text{-}H_2.$$

An example of this is the decomposition of lactic acid by the reductase of yeast, in the presence of methylene blue (M) as a hydrogen acceptor, as shown by the equation:

$$CH_3\text{-}CHOH\text{-}COOH \text{ (lactic acid)} + M =$$
$$CH_3\text{-}CO\text{-}COOH \text{ (pyrotartaric acid)} + MH_2.$$

The pyrotartaric acid produced is decomposed by carboxylase, into acetic aldehyde and carbon dioxide.[4]

If it is granted that reduction takes place with the participation of water, then the hydrogen of the water must unite with the acceptor of hydrogen, while

[1] **Grüss, J.,** Untersuchungen über die Atmung und Atmungsenzyme der Hefe. Zeitsch. ges. Brauwesen 27: 686–692, 699–704, 721–724, 734–739, 752–755, 769–772. 1904. **Palladin,** 1908. [See note 1, p. 168.]

[2] **Palladin, V. I. (W.),** and **L'vov, S. D.,** Sur l'influence des chromogènes respiratoires sur la fermentation alcoolique. [Text in Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, 7: 241–252. 1913. **Palladin, W.,** and **L'vov, Sergius,** Ueber die Einwirkung der Atmungschromogene auf die alkoholische Gärung. Zeitsch. Gärungsphysiol. 2: 326–337. 1913.

[3] **Palladin, V. I. (W).,** Sur le rôle des pigments respiratoires dans le respiration des plantes et les animaux. [Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg, *VI*, 6: 437–451. 1912. **Palladin, W.,** Ueber die Bedeutung der Atmungspigmente in den oxydationsprocessen der Pflanzen und Tiere. Zeitsch. Gärungsphysiol. 1: 91–105. 1912.

[4] **Palladin, Sabanin** and **Lochinovskaia,** Bull. Acad. Imp. Sci. St.-Pétersbourg, 1915. P. 701.*

[f] This and the four following paragraphs are translated from separate pages in Russian, received from Prof. Palladin. For another statement of these considerations and a report of some later work; see: **Palladin, W.,** and **Sabinin, D.,** The decomposition of lactic acid by killed yeast. Biochem. jour. 10: 183–196. 1916.—*Ed.*

the oxygen unites with the substance being oxidized, reacting with it either by the splitting off of hydrogen to form water or by some other oxidizing reaction. An example of a reaction in which water participates is furnished by the work of Wieland[1] on the oxidation of alcohol to form acetic acid by living or dead acetic bacteria in an oxygen-free atmosphere but in the presence of methylene blue (M). This reaction is represented by the equation:

$$CH_3CH_2OH \text{ (ethyl alcohol)} + H_2O + 2\,M = CH_3COOH \text{ (acetic acid)} + 2\,M\text{-}H_2.$$

It is possible, also, for water to be formed as a result of the union of hydrogen with an acceptor of hydrogen; for example, with potassium nitrate as acceptor, as represented by the equation:

$$R\text{-}H_2 + KNO_3 = R + KNO_2 + H_2O.$$

It follows that, after reduction, the molecule of the acceptor of hydrogen may become either richer by two atoms of hydrogen (methylene blue) or poorer by one atom of oxygen (potassium nitrate).

The action of reductase, in causing anerobic oxidation by means of the splitting off of hydrogen, may be accompanied by the production of carbon dioxide. Thus Bredig and Sommer[2] showed (see page 200) that, in the presence of a catalyzer and of methylene blue, formic acid is decomposed into carbon dioxide and hydrogen:

$$HCO_2H \text{ (formic acid)} + M = CO_2 + M\text{-}H_2.$$

Until recently the presence of reductase in plants was determined on the basis of the effect produced upon various acceptors of hydrogen. If no effect on these acceptors was observed, reductase was inferred to be absent, but this is not correct. In addition to a hydrogen acceptor there must be present a substance that may be oxidized, in order that the reductase may act. This was shown by Harden and Norris,[3] who found that reductase makes itself evident, in the dried yeast of Lebedev, only after the addition of both an oxidizer and a reducer.

Various bacteria and moulds (*e.g.*, the Mucoraceæ), as well as yeasts, produce alcoholic fermentation. Moulds generally form thick masses of mycelium upon the surface of the substratum and usually absorb considerable oxygen from the air. If the mycelium of such a mould is submerged in a fermentable liquid, alcoholic fermentation occurs, and the further development of the mycelium in the liquid is very characteristic. The long hyphæ divide to form cells that are very similar to those of yeast. It has recently been shown that the most active of these mucor yeasts produce alcoholic fermentation even in the presence of an abundance of oxygen[4] just as do ordinary yeasts.

[1] **Wieland, Heinrich,** Ueber den Mechanismus der Oxydationsvorgänge. Ber. Deutsch. Chem. Ges. 46^{III}: 3327–3342. 1913.

[2] **Bredig** and **Sommer,** 1910. [See note 2, p. 200.]

[3] **Harden, Arthur,** and **Norris, Roland Victor,** The reducing enzymes of dried yeast (Lebedeff) and of rabbit muscle. Biochem. jour. 9: 330–336. 1915.

[4] **Kostytschew, S.,** Untersuchungen über die Atmung und alkoholische Gärung der Mucoraceen. Centralbl. Bakt. *II*, 13: 490–503. 1904. **Wehmer, C.,** Versuche über Mucorineengärung. *Ibid. II*, 14: 556–572. 1905. **Idem,** same title. *Ibid. II*, 15: 8–19. 1906.

§3. **Other Kinds of Fermentation.**—Lactic acid fermentation (the souring of milk) is caused by *Bacillus lactici acidi*, which has the form of small paired rods from 1.0 to 1.7 micra long and from 0.3 to 0.4 micron broad. Many other bacteria are able to produce lactic acid fermentation; such as *Bacterium lactis acidi*, *Bacillus lactis acidi*, *Bacterium limbatum lactis acidi*, *Micrococcus lactis acidi*, *Sphærococcus lactis acidi*, *Streptococcus acidi lactici* and *Bacillus acidificans longissimus*.

The process of lactic acid fermentation is represented by the following equation:

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} = \underset{\text{Lactic acid}}{4\ C_3H_6O_3}.$$

This fermentation occurs when milk is simply exposed to a temperature of from 35° to 42°C. for a short time. The process stops when a certain amount of acid has accumulated, but if the acidity thus produced is neutralized with calcium carbonate fermentation begins again. Some acetic acid and other volatile acids usually occur, as well as lactic acid, the amount of these being dependent both upon the kind of bacterium and upon the composition of the nutrient medium. Besides lactose, other kinds of sugars, such as cane sugar, fructose and maltose, can be fermented to lactic acid if the proper kind of bacteria is used.

Lactic acid may also be obtained if a mixture of 100 g. of cane sugar and 10 g. of casein or old cheese, in a liter of water saturated with calcium carbonate, is allowed to stand in an open vessel at a temperature of from 35° to 40°C., with occasional shaking. After fermentation has ceased the liquid is evaporated and calcium lactate is deposited, from which free lactic acid is obtained by decomposing the lactate with sulphuric acid. The optically inactive variety of lactic acid is obtained in this process, but in some cases the optically active isomers arise. When *Micrococcus acidi paralactici* acts in a medium containing sugar, appreciable amounts of the dextro-rotatory paralactic acid are formed; *Bacillus acidi levolactici* forms the levo-rotatory acid. The different powers possessed by different bacteria to form optically active isomers of lactic acid may be used in identifying related species of these organisms; thus, *Bacterium coli commune* decomposes grape sugar, giving dextro-lactic acid, but *Bacillus typhi abdominalis* produces levo-lactic acid under the same conditions. Lactic acid bacteria have been widely applied in the industries; for example, Berlin white beer is obtained by the action of these forms.

Butyric acid fermentation is produced by the bacterium, *Clostridium butyricum*, which has recently been shown to consist of a mixture of at least three different species. There are also many other bacteria that produce butyric acid. Butyric acid fermentation occurs in the complete absence of oxygen, and both hydrogen and carbon dioxide always arise as gaseous products of the process. The reaction is represented by the following equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Hydrogen}}{2\ H_2} + \underset{\text{Carbon dioxide}}{2\ CO_2} + \underset{\text{Butyric acid}}{C_4H_8O_2}.$$

When lactic acid is fermented instead of sugar the reaction becomes the following:

$$\underset{\text{Lctica acid}}{2\,C_3H_6O_3} = \underset{\text{Hydro-gen}}{2\,H_2} + \underset{\text{Carbon dioxide}}{2\,CO_2} + \underset{\text{Butyric acid}}{C_4H_8O_2}.$$

To obtain butyric acid fermentation a mixture is prepared containing 2 l. of water, 100 g. of potato starch (or dextrin), 1 g. of ammonium chloride and other nutrient salts, and 50 g. of chalk, and this is allowed to stand at 40°C.

Numerous bacteria are known that cause different kinds of fermentation, but an account of each separate process is not here possible. It should be mentioned, however, that these various bacteria produce numerous and diverse chemical reactions, far surpassing the well-known chemical reagents in sensitiveness and specificity.[1]

§4. Plant Respiration.[2]—Ingen-Housz (1779) was the first to demonstrate that living plants respire. In repeating the experiments of Priestley upon the improvement of air by plants, Ingen-Housz showed that this alteration of the air is accomplished only by the green parts of plants and that it occurs only in sunlight; the non-green parts of plants are like animals, as far as their effect upon the air is concerned, and unilluminated green plant parts also act in the same way, to "poison" the air. (See p. 2.) This poisoning of the air is due to the elimination of carbon dioxide and is the result of respiration. The first exact experimentation upon plant respiration was carried out by Saussure in 1804.

The influence of external conditions upon the respiratory activity of plants has received the attention of many investigators. The effect of temperature has been studied with unusual care,[3] thermostats of various kinds being used to keep the temperature constant during the period of an experiment. The rate of gaseous exchange is nearly proportional to the temperature, for medium temperatures, but a maximum rate is reached at about 40°C. and further rise in temperature is without influence upon this rate, which remains constant until death supervenes. The value of the respiratory ratio (the amount of carbon dioxide given off divided by the amount of oxygen absorbed in a unit of time, $\frac{CO_2}{O_2}$) reaches a minimum at about 10° or 15°C., and increases with higher as well as with lower temperatures, the increase being more rapid in the first case. This is illustrated by the following table of experimental results, taken from the work of Purievich.[4]

[1] **Oméliansky, W.**, De la méthode bactériologique dans les recherches de chimie. Arch. sci. biol. St. Pétersbourg **12**: 224–247. 1907.

[2] **Palladin**, 1909. [See note 2, p. 204.] **Czapek, Friedrich**, Die Atmung der Pflanzen, Ergeb. Physiol. 9: 587–613. 1910. **Nicolas, G.**, Recherches sur la respiration des organes végétatifs des plantes vasculaires. Ann. sci. nat. Bot. *IX*, **10**: 1–113. 1909. **Reinitzer, Fr.**, Ueber Atmung der Pflanzen. (Antrittsrede.) 17 p. Graz, 1909. Rev. in: Bot. Centralbl. **115**: 52. 1910.

[3] **Wolkoff, A. v.**, and **Mayer, Adolf**, Beiträge zur Lehre über die Athmung der Pflanzen. Landw. Jahrb. 3: 481–527. 1874. **Bonnier, Gaston**, and **Mangin, Louis**, Recherches sur la respiration et la transpiration des champignons. Ann. sci. nat. Bot. *VI* 17: 210–305. 1884. **Kuijper, J.**, Ueber den Einfluss der Temperatur auf die Atmung der höheren Pflanzen. Recueil trav. bot. Néerland. 7 131–240. 1910.

[4] Puriewitsch, 1893. [See note 2, p. 188.]

Plant	Temperature, *deg. C.*	Respiratory Ratio, $\frac{CO_2}{O_2}$
Sedum hybridum	2–4	0.45
	10–12	0.37
	25–26	0.48
Pelargonium zonale	4–5	0.75
	12–14	0.54
	34–35	0.95

Temperature fluctuations themselves exert great influence upon plant respiration, aside from the effect produced by altered temperature. Palladin[1] exposed three similar lots of tips of etiolated bean seedlings to three different temperatures, respectively, and then brought them all to the same medium temperature and determined the rate of evolution of carbon dioxide in each case. The following table illustrates the kind of results obtained.

Previous Temperature, *deg. C.*	Relative Amounts of CO_2 Produced per Unit of Time, 18–22°C.	Average	Excess, Per Cent.
Medium, 17–20	54.5, 53.5, 55.0, 44.9, 58.1, 65.3, 59.8,	55.8	
Low, 7–12	89.8, 73.6, 80.2, 53.9, 78.9, 87.4, 82.9	78.1	40
High 36–37	81.4, ... 89.4, ...	85.4	53

The tips that remained at medium temperature formed the least carbon dioxide, but those that had been recently transferred from lower to higher or from higher to lower temperature produced much more of this gas.

A very peculiar influence of temperature upon the respiration and vital activity of *Aspergillus niger* was observed by A. Rikhter.[2] Frozen mycelium of this fungus, when allowed to thaw at room temperature, appeared to have been killed, and produced no trace of carbon dioxide. When the frozen filaments were transferred directly to a temperature of 30°C., however, this gas began to be given off. The rate of evolution of the gas increased gradually and spores were formed. This shows that freezing is not fatal, *per se;* death is of later occurrence, with the thawing of the organism, under unfavorable temperature conditions.

An indirect relation between light conditions and respiration was discovered by Borodin,[3] who found that the intensity of respiratory activity in leafy twigs gradually decreases after the twigs are placed in darkness, and rises again after they have been once more illuminated. These phenomena may be interpreted as follows: Carbohydrates are necessary for respiration and are gradually used up

[1] **Palladin, W.,** Influence des changements de température sur la respiration des plantes. Rev. gén bot. **11**: 241–257. 1899.

[2] **Richter, A.,** Zur Frage über den Tod von Pflanzen infolge niedriger Temperatur. (Kälteresistenz von Aspergillus niger.) Centralbl. Bakt. *II*, **28**: 617–624. 1910.

[3] **Borodin, J. P.,** Physiologische Untersuchungen über die Atmung beblätterter Sprosse. St. Petersburg, 1876.* [**Idem,** Sur la respiration des plantes pendant leur germination. Florence, 1875.]

during the period of darkness, so that respiration is at length retarded because of lack of material. When the plants are returned to the light the supply of available carbohydrates is again increased and the respiratory process returns to its usual rate. Such an interpretation finds further support in the observation that the change from darkness to light is accompanied by an acceleration in the evolution of carbon dioxide only when the light contains the less refrangible wave-lengths (which are especially active in photosynthesis), and when the surrounding air is supplied with carbon dioxide (without which photosynthesis cannot occur).

Bonnier and Mangin[1] state that there is also a direct influence of light upon plant respiration, but that this is very slight. If plants are placed alternately in darkness and in light a retarding effect of light is observed, and this bears no relation to the photosynthetic process, since it is demonstrable in plants without chlorophyll. The value of the respiratory ratio is independent of light.[g]

Maksimov[2] came to the conclusion that the effect of light upon the respiration of *Aspergillus niger* varies with the age of the culture and with the nature of the nutrient medium. He found that light exerted no influence upon the respiration of young, well-nourished cultures, but that the respiration of old cultures was increased by illumination. The stimulating effect became more marked if the culture was deficient in nutrient material. Levshin,[3] however, could observe no influence of diffuse light upon the rate of respiration in various fungi.

The partial pressure of oxygen in the surrounding atmosphere also influences plant respiration. In this case, also, the value of the respiratory ratio does not change.

According to the results of Kosinski[4] and Palladin,[5] the concentration of the nutrient solution exerts great influence upon the rate of respiration. If plants are transferred from a more concentrated to a more dilute solution respiration becomes more active, and a change in the opposite direction decreases respiratory activity. Thus, 100 g. of etiolated bean leaves, with their petioles dipping into a cane-sugar solution that was altered in concentration from time to time, gave the following mean hourly rates of evolution of carbon dioxide, for the different exposure periods.

[1] **Bonnier, Gaston,** and **Mangin, Louis,** Recherches sur la respiration des tissus sans chlorophylle. Ann. sci. nat. Bot. *VI*, **18**: 293–382. 1884.

[2] **Maximow, N. A.,** Ueber den Einfluss des Lichtes aud die Atmung der niederen Pilze. Centralbl. Bakt. *II*, **9**: 193–205, 261–272. 1902.

[3] **Löwschin, A.,** Zur Frage über den Einfluss des Lichtes auf die Atmung der niederen Pilze. Beih. Bot. Centralbl. **23**: 54–64. 1908.

[4] **Kosinski, Ignacy,** Die Athmung bei Hungerzuständen und unter Einwirkung von mechanischen und chemischen Reizmitteln, bei Aspergillus niger. Jahrb. wiss. Bot. **37**: 137–204. 1902.

[5] **Palladin, W.,** and **Komleff, A.,** Influence de la concentration des solutions sur l'énergie respiratoire et sur la transformation des substances dans les plantes. Rev. gén. bot. **14**: 497–516. 1902.

[g] The respiratory activity of plant parts containing chlorophyll is of course difficult to study as long as light is present, because of the fact that photosynthesis reverses the respiration process, as far as the absorption of oxygen and the elimination of carbon dioxide is concerned. In this connection, as well as with regard to the influence of light on respiration itself, see: **Spoehr, H. A.,** Photochemical processes in the diurnal deacidification of the succulent plants. Biochem. Zeitsch. **57**: 95–111. 1914. **Idem,** Variations in respiratory activity in relation to sunlight. Bot. gaz. **59**: 366–386. 1915.—*Ed.*

Concentration of Medium	Period of Exposure	CO_2 Produced per Hour	Change in Respiratory Rate
per cent.	*days*	*mg.*	*per cent.*
15	3	122.7	
25	3	79.4	−32.5
50	1	69.7	−12.2
0	1	154.0	+120.9

Zaliesskii[1] found that if the bulbs of Gladiolus are immersed in water for a short time their respiratory activity is considerably increased.

Changes in concentration of the nutrient solution affect the value of the respiratory ratio. Purievich[2] obtained the following values of this ratio for *Aspergillus niger* with different concentrations of cane-sugar solution.

Concentration of the medium, *per cent*	1	5	10	20	25
Respiratory ratio, $\left(\frac{CO_2}{O_2}\right)$	0.85	0.96	1.04	0.93	0.73

Respiration is influenced by various toxic substances.[3] Morkovin[4] studied this effect in the case of various alkaloids, glucosides, alcohols and other substances, such as ethyl ether, formaldehyde and paraldehyde, and found that these increase respiratory activity when present in very weak concentration. For example, of two similar groups of shoots of etiolated bean seedlings one group was grown in cane-sugar solution, and the other in the same solution with the addition of 1 per cent. of isobutyl alcohol. Without the poison, 100 g. of shoots produced 65.0 mg. of carbon dioxide per hour during the first twenty-four hours of the experiment, and 72.4 mg. per hour during the first thirty-seven hours. With the poison, 191.7 mg. of carbon dioxide was produced per hour for the first twenty-four hours and 124.5 mg. per hour for the first thirty-seven hours. Isobutyl alcohol, in this concentration, is thus seen to exert a definitely accelerating effect upon respiration. Zaliesskii[5] has shown that ether accelerates respiration in resting plant organs to a marked degree; in the case of Gladiolus bulbs exposed to an atmosphere containing ether, respiration is first increased, but later decreases to below the normal rate.

Wounding markedly increases the rate of respiration.[6] In one experiment

[1] **Zaliesskii, V.,** Influence de l'excitation sur la respiration des plantes. [Russian, French sub-title only.] Mém. Inst. Agron. et Forest. Novo-Alexandria **15**[2]: 1–41. 1902. [Parts of vol. are separately paged.]

[2] **Puriewitsch, K.,** Physiologische Untersuchungen über Pflanzenatmung. Jahrb. wiss. Bot. **35**: 573–610. 1900.

[3] **Palladin, W.,** Ueber die Wirkung von Giften auf die Atmung lebender und abgetöteter Pflanzen, sowie auf Atmungsenzyme. Jahrb. wiss. Bot. **47**: 431–461. 1910.

[4] **Morkowin, N.,** Recherches sur l'influence des anesthétiques sur la respiration des plantes. Rev. gén. bot. **11**: 289–303, 341–352. 1899. **Idem,** Recherches sur l'influence des alcaloides sur la réspiration des plantes. *Ibid.* **13**: 109–126, 177–192, 212–226, 265–275. 1901.

[5] **Zaliesskii,** 1902. [See note 1, this page.)

[6] **Stich, Conrad,** Die Athmung der Pflanzen bei verminderter Sauerstoffspannung und bei Verletzungen. Flora **74**: 1–57. 1891. P. 15. **Pfeffer, W.,** Ueber die Steigerung der Athmung und der Wärmeproduction nach Verletzung lebensthätiger Pflanzen. Ber. ü. d. Verh. d. K. Sächs. Ges. Wiss. Leipzig (Math.-phys. Cl.) **48**: 384–389. 1896. **Smirnoff,** Influence des blessures sur la respiration normale et intramoléculaire (fermentation) des bulbes. Rev. gén. bot. **115**: 26–38. 1903.

300 g. of uninjured potato tubers produced from 1.2 to 2 mg. of carbon dioxide per hour. After this rate had been determined each tuber was quartered, and the pieces were left at the same temperature and in the same surroundings as before. For the second hour after cutting, the rate of evolution of carbon dioxide was 9 mg.; for the fifth, 14.4 mg.; for the tenth, 16.8 mg.; and for the twenty-eighth, 18.6 mg. Then the rate began to decrease. For the fifty-first hour after cutting it was 13.6 mg., after four days it was 3.2 mg., and after six days it had fallen to 1.6 mg., the original average rate obtained before wounding.

Phosphates,[h] which markedly accelerate alcoholic fermentation, have the same effect upon respiration, which, as has been seen, is related to alcoholic fermentation.[1] These salts thus accelerate both the anaerobic and the oxidation phase of the respiratory process.[2]

The rate of plant respiration depends, furthermore, upon various internal conditions, within the organism. In the first place may be mentioned the relation between respiration and growth. The more rapidly a plant grows, the more oxygen does it absorb and the more carbon dioxide does it give off. As will appear in the sequel (page 247), all plants exhibit the so-called grand period of growth, which may be represented by the grand curve of growth. A germinating seedling grows slowly at first, but with increasing rapidity as it becomes older, until a maximum growth rate is attained, after which growth proceeds more and more slowly. The intensity of respiration is found also to be very low during the early stages of growth; with increasing growth rates the respiratory process is accelerated and this also reaches a maximum intensity and then declines. Thus may be constructed a grand curve of respiration, the form of which is practically identical with that of the grand curve of growth. This grand curve of respiration was first shown by A. Mayer, who measured the oxygen absorbed. Like results were obtained by Borodin and Rischavi,[3] who determined the amount of carbon dioxide eliminated.

The value of the respiratory ratio $\left(\frac{CO_2}{O_2}\right)$ does not remain constant during seed germination. Bonnier and Mangin[4] showed that this value is unity for the first phase of germination, but that it becomes smaller with increasing growth rates. Palladin[5] came to a similar conclusion from a study of the value of the respiratory ratio for actively growing internodes cut from the stems of various kinds of plants. In all these experiments the value of the ratio was less than unity, which shows that growing organs absorb more oxygen than they give off

[1] **Iwanoff, Leonid,** Ueber die Wirkung der Phosphate auf die Ausscheidung der Kohlensäure durch Pflanzen. Biochem. Zeitsch. **25**: 171–186. 1910. **Iwanoff, Nicolaus,** Die Wirkung der nützlichen und schädlichen Stimulatoren aud die Atmung der lebenden und abgetöteten Pflanzen. *Ibid* **32**: 74–96. 1911.

[2] **Zaleski, W.,** and **Reinhard, A.,** Zur Frage der Wirkung der Salze auf die Atmung der Pflanzen und auf die Atmungsenzyme. Biochem. Zeitsch. **27**: 450–473. 1910.

[3] **Mayer, A.,** Ueber den Verlauf der Athmung beim keimenden Weizen. Landw. Versuchsstat., **18**: 245–279. 1875. **Borodin,** 1875. [See note 3, p. 211.] **Rischavi, L.,** Einige Versuche über die Athmung der Pflanzen. Landw. Versuchsstat. **19**: 321–340. 1876.

[4] **Bonnier** and **Mangin,** 1884. [See note 1, p. 212.]

[5] **Palladin, W.,** Athmung und Wachsthum. (Auszug aus einer russisch erscheinenden Arbeit.) Ber. Deutsch. Bot. Ges. **4**: 322–328. 1886.

[h] This paragraph is omitted in the 7th Russian edition.—*Ed.*

in the carbon dioxide eliminated. In such organs cellulose is accumulating and asparagin is being formed, and both of these processes are dependent upon the assimilation of oxygen.

Respiration is closely related to all of the other processes occurring in living cells. The relation of fat and carbohydrate content to the respiration of germinating seeds may serve as an illustration of this. Many studies agree in showing that the germination of fatty seeds exhibits respiratory ratio values that are exceptionally low. It is thus suggested that the germinal activity of such seeds is connected with a fixation of oxygen. It has been pointed out (page 191) that the loss during the germination of fatty seeds is made up only of carbon and hydrogen, while the amount of oxygen in the seeds increases. This becomes clear in connection with the fact that the respiration of these seeds involves the oxidation of fats, whose oxygen content is much smaller than that of cabohydrates. Therefore, the value of the ratio $\frac{CO_2}{O_2}$ must be markedly less than unity in this case. The complete oxidation of triolein may be represented by the following equation;

$$\underset{\text{Triolein}}{C_3H_5O_3\,(C_{18}H_{33}O)_3} + \underset{\text{Oxygen}}{80O_2} = \underset{\text{Carbon dioxide}}{57\,CO_2} + \underset{\text{Water}}{52\,H_2O}.$$

Here the value of the oxidation ratio is $\frac{57}{80}$, which is less than unity. Polovtzov[1] has shown that fatty seeds germinating in cane-sugar solution produce a direct oxidation of sugar, the respiratory ratio being equal to unity in this case.

The gas exchange accompanying respiration in ripening fruits that have oily seeds, after the fats have begun to accumulate, presents a very different picture. The formation of oils from carbohydrates (the direct products of photosynthesis) is possible only with the elimination of the superfluous oxygen. Thus, the rate of carbon dioxide production increases in these ripening fruits, without any corresponding increase in the rate of oxygen absorption, and the value of the respiratory ratio becomes *greater* than unity. An experiment with ripening poppy fruits[2] showed a rate of oxygen absorption of 21.72 cc. while the corresponding rate of carbon dioxide production was 32.62. Thus, $\frac{CO_2}{O_2} = 1.5$, which is greater than unity.

§5. Apparatus for Measuring Plant Respiration.[3]—In respiration studies it is necessary to measure one or both of the gases involved. When the determination of the rate of elimination of carbon dioxide is sufficient, Pettenkoffer tubes (Fig. 91) are serviceable. These are glass tubes about 1.5 cm. in diameter and about a meter long, filled with titrated baryta water [preferably barium hydroxide dissolved in an aqueous solution of barium chloride] and supported in an oblique position. A water aspirator is used to produce a slow current of

[1] **Polovtsov, V.,** Études sur la respiration des plantes. Mém. Acad. Imp. Sci. St.-Pétersbourg VIII, 12[7]: 1–69. 1902.

[2] **Godlewski, Emil,** Beiträge zur Kenntniss der Pflanzenathmung. Jahrb. wiss. Bot. 13: 491–543. 1882.

[3] **Palladin, W., and Kostytschew, S.,** Methoden zur Bestimmung der Athmung der Pflanzen. Abderhalden's Handbuch 3: 479–515. 1910

air, which enters the plant chamber (*a*, Fig. 91) after having been freed of carbon dioxide through the action of soda lime. From the plant chamber the air passes into the lower end of the Pettenkoffer tube, forming small bubbles which ascend slowly through the baryta water. The air, again freed of carbon dioxide, passes out to the aspirator from the upper end of the tube. Since the aspirator would usually produce a more rapid air stream than can be passed through the Pettenkoffer tube, a pressure regulator (*b*, Fig. 91) is introduced, which also prevents too great rarification of the air in the plant chamber. The carbon dioxide produced by the plants is precipitated in the tube as barium carbonate. After a suitable time the air stream is turned into a second Pettenkoffer tube and the solution is removed from the first and titrated [with standard oxalic acid solution and phenolphthalein as indicator]. Thus the amount of unprecipitated barium hydroxide that remains is determined, and a simple calculation gives the weight of the carbon dioxide produced by the plants during the given period. The temperature of the plant chamber is maintained constant by immersing it in a large vessel of water which is warmed as necessary.

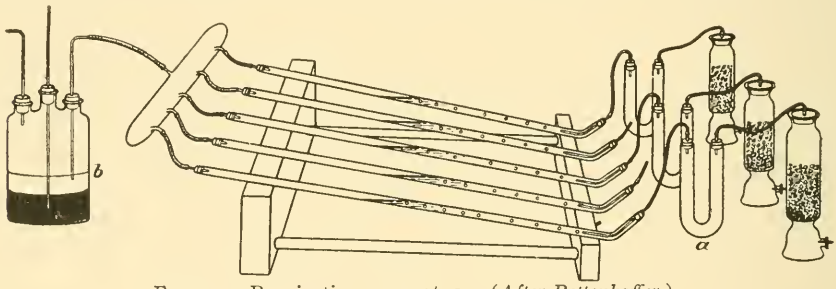


FIG. 91.—Respiration apparatus. (*After Pettenkoffer.*)

The amount of oxygen absorbed by a plant may be measured by means of the apparatus of Wolkoff and Mayer (see note 3, p. 210), which consists essentially of a large inverted U-tube with one arm broad and the other narrow and graduated for volume readings. In the broad arm of this tube are placed the seedlings, etc., to be studied, and also a small, open vessel of potassium hydroxide solution, and the larger opening is tightly closed with a glass stopper. The other, narrow arm of the tube is closed by dipping into mercury below. The carbon dioxide produced by the plant is absorbed by the potassium hydroxide solution and the volume of the oxygen absorbed is measured by the rise of the mercury meniscus in the narrow, graduated arm.

For the simultaneous determination of the oxygen absorbed and the carbon dioxide given off, the apparatus of Bonnier and Mangin may be employed (Fig. 92). The bell-jar, *A*, serves as plant chamber, into which air passes through the tube *a*, having first been freed of carbon dioxide by bubbling through potassium hydroxide solution in the wash-bottle, *F*. A vessel of water in the chamber keeps the atmosphere moist. The chamber is first filled with air that has been freed from carbon dioxide, suction being applied through tube *b*, by means of an aspirator. Then the two cocks, *r* and 2, are closed. From time to time a

gas sample is removed from the plant chamber and analyzed, the removal of this sample being accomplished as follows: The three-way cock R is so set as to bring the tube b into communication with the container l, after which the similar container l' is lowered, so that some mercury flows fron l to l', thus drawing air from the plant chamber into l. Then the cock R is reset so that l communicates with tube d and the sample tube beyond, and the container l' is again raised, thus forcing into the sample tube some of the gas that has just been removed from the plant chamber.

The volume of the gas in the plant chamber is determined as follows: Some gas is removed and its volume (V) is determined at atmospheric pressure (H). If p is the gas pressure in the apparatus before, and p' is the pressure after, the removal of this gas (these pressures being determined by means of the mano-

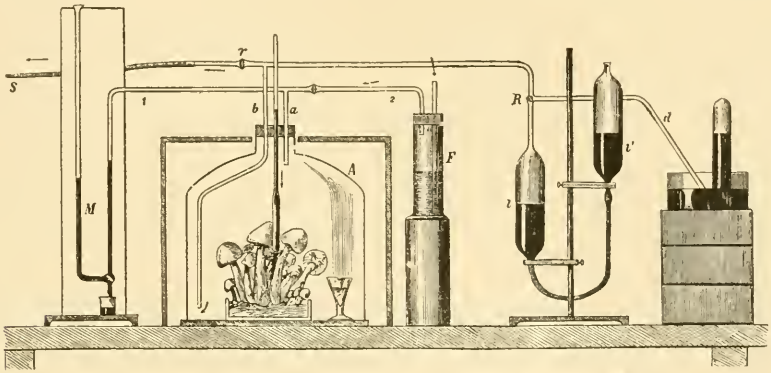


FIG. 92.—Respiration apparatus. (*After Bonnier and Mangin.*)

meter, M), then the original gas volume (X) contained in the chamber is found from the equation:

$$X = \frac{VH}{p - p'}.$$

If the absolute amounts of oxygen absorbed and of carbon dioxide given off are not important, then the determination of the total gas volume is not required. In such a case the value of the ratio $\frac{CO_2}{O_2}$ is derived from the proportions of these two gases found in the samples taken at the beginning and end of the experiment.

§6. **Formation of Water during Respiration.**—During germination in darkness all seeds lose an appreciable amount of hydrogen, in the form of the water vapor produced by the respiratory process. Very few direct determinations of respiration water are available. Liaskovskii[1] studied the formation of water during the germination of pumpkin seeds. The seeds were germinated under a bell-jar, through which a current of air was drawn, the entire apparatus being weighed from time to time. The amount of water produced by the respira-

[1] **Liaskovskii,** 1874. [See note 2, p. 191.]—Also, in this connection, see: **Babcock,** 1912. [See note 3, p. 189.]—[Babcock deals with the water of respiration in insects (such as the common clothes moth, which lives on dry wool) as well as in germinating seeds.—*Ed.*]

tory process was obtained from five measured values, as follows: the total weight of the apparatus at the beginning (A) and at the end (B) of the experiment, the dry weight of the seeds before (m) and after germination (n) and the amount of water actually given off (O). The water eliminated during the experiment, was collected in calcium chloride tubes. Supposing that the weight of the empty apparatus (S) and the air therein contained (U) suffered no change during the experiment, the amount of water formed by respiration can be easily calculated from these data. At the beginning of the experiment the amount of water contained in the seeds and in the whole system is equal to $A - S - U - m$, which may be designated as X. At the end of the experiment the amount of water in the system, aside from the absorption tubes, is equal to $B - S - U - n$, which may be called Y. The amount of water retained in the absorption tubes (O) is to be added to Y, to give the total amount present at the end of the experiment. The difference between the amount present at the beginning and that at the end, is of course the amount produced by respiration. If this difference is represented by Z, then we have:

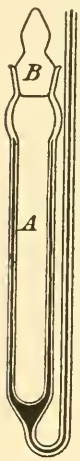


FIG. 93.—Calorimeter. (*After Regnault.*)

$$Z = Y + O - X = B - n + O - A + m.$$

The results obtained by Liaskovskii may be summarized as follows:

1. In the early stages of germination very little water, or none at all, is produced.

2. With higher temperatures the production of water is relatively less than with lower temperatures.

3. There is no constant relation between the amount of carbon dioxide and that of hydrogen given off.

The low rate of water formation in the early stages of germination may be due to the fact that various hydrolytic processes are very active at this time. How great may be the amount of water fixed by hydrolytic changes will be brought out by the experiments of Bonnier, to be described in the next following section.

§7. **Liberation of Heat During Respiration.**—The internal temperature of the plant body is generally about the same as that of the surrounding air, and it is only by very careful experimentation that it is possible to demonstrate slight differences. The temperature of growing shoots usually exceeds that of the surrounding air by not over 0.3°C. Only two periods in the life of the plant exhibit an appreciable production of heat, that of seed germination and that of flowering.[i] The temperature of germinating seeds is from 7 to 20°C. higher than that of the surrounding air, and the difference is still more pronounced in the case of opening flower buds.[1] A temperature of 49° has been observed in the flowering spadix of some of the Aroideæ, when that of the surrounding air was only 19°. The rise of temperature is here concomitant with an accelerated rate of oxygen absorption.

[1] Kraus, Gregor, Physiologisches aus den Tropen. *III.* Über Blüthenwärme bei Cycadeen, Palmen und Araceen. Ann. Jard. Bot. Buitenzorg 13: 217–275. 1896.

[i] Large leaf buds of deciduous trees, as they open in the spring, should also be mentioned here. Expanding buds of the horse-chestnut (Æsculus) furnish an example.—*Ed.*

Bonnier[1] has carried out extensive researches upon the production of heat during seed germination, using either a calorimeter of the Berthelot type or the modified thermo-calorimeter of Regnault. The latter apparatus (Fig. 93) consists essentially of a mercury thermometer the bulb of which is expanded to form the wall of a chamber (*A*), the latter being closed by a stopper (*B*). The plants or plant parts to be studied are placed in this chamber and their temperature is directly read on the thermometer scale. In some of Bonnier's experiments analyses of the gas contained in the chamber were also carried out.

Pea seeds placed in this calorimeter and allowed to grow until the cotyledons had disappeared produced the following amounts of heat per minute, per kilogram of seeds, at different stages of their development.

Stage of Development, Pea	Heat Produced per Minute, *gram-calories*
1. Soaked seeds	9
2. Seedlings with roots 5 mm. long	125
3. Seedlings with roots 50–60 mm. long	75
4. Seedlings with green stem abuut 20 mm. long	60
5. Seedlings with cotyledons withering	22
6. Seedlings from which cotyledons have fallen	6

This experiment shows that the rate of heat production varies with the development of the plant, the maximum rate occurring with a very early stage of germination.

If the rates of heat liberation for the different developmental stages are calculated from the rates of the elimination of carbon dioxide and of the absorption of oxygen, the results do not agree with the corresponding ones determined calorimetrically, as is clear from the following table, which gives the rates of heat production per kilogram of barley seeds per minute.

Stage of Development, Barley	Heat Produced per Minute		Respiratory Ratio Value $\left(\frac{CO_2}{O_2}\right)$
	Calorimetrically Determined	Calculated	
	gram-calories	*gram-calories*	
1. Soaked seeds	5	3	1.00
2. Root primordia showing	62	45	0.65
3. Main root 3 mm. long	40	31	0.80
4. End of germination	15	12	0.95
5. Leafy stems	0	3	1.00

The amounts of heat actually produced in germination markedly exceed the corresponding calculated amounts, and it is therefore evident that exothermic reactions other than that of oxidation occur during germination, especially in the earlier stages. Among such reactions are to be included starch inversion

[1] **Bonnier, Gaston,** Recherches sur la chaleur végétale. Ann. sci. nat. Bot. *VII*, **18**: 1–35. 1893.

and other hydrolytic processes. (See last paragraph of the next preceding section, p. 218.)

More mature, growing stems are seen to be different from germinating seeds in this regard; while the calculation leads us to expect a rate of heat production here of 3 g.-cal. per minute, the calorimetric determination shows that no heat is liberated at all. In this case the energy is not set free as heat but must be considered as taking the form of work, the accomplishment of which is a necessity in every active cell. Work and heat are merely different modifications of the same thing, *energy*—just as the yellow and red varieties of phosphorus, or the diamond and amorphous carbon, are simply different forms of matter.[1]

Some thermo-chemical considerations are of interest in this connection. The heat of formation of carbon dioxide is 97,600 g.-cal. per gram-molecule, and that of the CO_2 used for a gram-molecule of carbohydrate (employing the empirical formula for starch, $C_6H_{10}O_5$) is 97,600 × 6, or 585,600 g.-cal.[i] Experiment shows that the heat of formation of a gram-molecule of carbohydrate to be actually 667,000 g.-cal., however, and the excess (81,400 g.-cal., the so-called heat effect) is the amount of heat corresponding to the formation of a gram-molecule of starch from C and H_2O. The heat of combustion of starch is thus made up of the heat of formation of 6 molecules of carbon dioxide and that of the combination of 5 molecules of water with these. When carbohydrates are completely oxidized in the animal body there is the same excess of heat (81,400 g.-cal.) above that of the oxidation of the carbon in the carbohydrate molecule. This explains the fact—not otherwise to be understood—that the animal body produces an apparent excess of heat above that which is calculated from the amount of carbon dioxide eliminated,[2] or from the quantity of oxygen absorbed, this calculation being based simply on the oxidation of carbon to carbon dioxide. In the concrete case just considered, the calculated heat of combustion of starch (585,600) is about six-sevenths of the value obtained by direct observation (667,000). The differences encountered in Bonnier's experiments are so great, however (see the table given above), that they are not to be referred simply to the heat effect. It is strongly suggested that reactions occur in seed germination whereby heat is liberated without the occurrence of oxidation.

The experiment of Bonnier, above described, shows that the highest rate of heat production occurs when the respiratory ratio, $\frac{CO_2}{O_2}$, assumes a minimum value and the rate of oxygen absorption is much accelerated.

§8. Anaerobic, or Intramolecular, Respiration.—When plants that usually require oxygen are placed in an oxygen-free atmosphere they do not die at once

[1] **Ostwald, Wilhelm,** Theoretische Chemie. Moscow, 1891. P. 73.*

[2] **Ostwald, Wilhelm,** 1891.* [See reference just given.]

[i] This number corresponds to the formation of 6 gram-molecules of carbon dioxide from carbon and oxygen, the hydrogen and oxygen of the starch molecule being considered simply as 5 molecules of water. In other words, $C_6H_{10}O_5$ is considered as though it were $6C + 5H_2O$. The hydrogen and oxygen of starch are not combined to form water, however, and, as is brought out in the next sentence of the text, the heat of formation of $C_6H_{10}O_5$ from $6C + 5H_2O$ is the excess there referred to. The German edition agrees with the 7th Russian edition in stating this excess as 82,300, instead of 81,400 g.-cal.—*Ed.*

but remain alive for a time, and the evolution of carbon dioxide continues.[1] Ethyl alcohol is usually formed also.[2] This *anaerobic*, or *intramolecular*, respiration is mainly the same as alcoholic fermentation.

Sometimes the amount of carbon dioxide produced with access of oxygen is the same as in the absence of oxygen, but such cases are rare; usually carbon dioxide production is considerably less when oxygen is not available.[3] The value of the ratio of the amount of carbon dioxide eliminated anaerobically to the amount given off in the same time in the presence of oxygen is given below for several plants.

Young seedlings of *Vicia faba* (Windsor bean)	1.197
Young seedlings of *Triticum vulgare* (wheat)	0.490
Young twigs of *Abies excelsa* (fir)	0.077
Young twigs of *Ligustrum vulgare* (privet)	0.816

The amount of carbon dioxide formed in anaerobic respiration is primarily dependent upon the carbohydrate content of the plant in question.[4] Etiolated bean leaves produce but very little carbon dioxide in the absence of oxygen, and die within two days. If they are previously kept with their petioles in sugar solution for some time, being then placed under anaerobic conditions, they produce much carbon dioxide and live much longer than when they are employed without the preliminary sugar treatment. After two days they are still alive and they afterwards become green if illuminated.

In anaerobic respiration, alcohol is formed only from carbohydrates. Seventy-one etiolated leaves of *Vicia faba*, which had been previously supplied with sugar as above, formed, without oxygen, 782.4 mg. of carbon dioxide and 724.6 mg. of alcohol, in twenty-five hours. The same number of similar leaves, not previously supplied with carbohydrate, but otherwise treated in the same way, gave off 256.8 mg. of carbon dioxide and 68.3 mg. of alcohol, in thirty hours. In the first case the ratio of the amount of carbon dioxide to that of alcohol produced is 100: 92.6, and in the second case the corresponding ratio is 100: 26.5. It should be added here that alcohol elimination in the second instance was confined to the first few hours of the experiment, before the limited amount of plastic carbohydrates that was present had been exhausted.[5]

[1] **Lechartier, G.,** and **Bellamy, F.,** Etude sur les gaz produits par les fruits. Compt. rend. Paris **69**: 356–360. 1869. **Idem,** De la fermentation des fruits. *Ibid.* **69**: 466–469. 1869. **Idem,** same title. *Ibid.* **75**: 1203–1206. 1872. **Pasteur, Louis,** Faits nouveaux pour servir à la connaissance de la théorie des fermentations proprement dites. *Ibid.* **75**: 784–791. 1872.

[2] **Godlewski, E.,** and **Polzeniusz, F.,** Ueber Alkoholbildung bei der intramolecularen Athmung höherer Pflanzen. (Vorläufige Mittheilung.) [Title also in Russian, text in German.] Bull. Int. Acad. Sci. Cracovie **1897**: 267–271. 1897. **Idem,** Ueber die intramoleculare Athmung von in Wasser gebrachten Samen und über die dabei stattfindende Alkoholbildung. [Title also in Russian and French, text in German.] *Ibid.* **1901**: 227–276. 1901. **Nabokich, A. J.,** Ueber die intramolekulare Atmung der höheren Pflanzen. Ber. Deutsch. Bot. Ges. **21**: 467–476. 1903. **Palladin, W.,** and **Kostytschew, S.,** Anaërobe Atmung, Alkoholgarung und Acetonbildung bei den Samenpflanzen. Zeitsch. physiol. Chem. **48**: 214–239. 1906. **Idem,** Ueber anaërobe Atmung der Samenpflanzen ohne Alkoholbildung. Ber. Deutsch. Bot. Ges. **25**: 51–56. 1907. **Stoklasa, Julius, Ernest, Adolf,** and **Chocensky, Karl,** Ueber die glykolytischen Enzyme im Pflanzenorganismus. Zeitsch. physiol. Chem. **50**: 303–360. 1906–1907.

[3] **Pfeffer, W.,** Ueber intramolekulare Athmung. Untersuch. Bot. Inst. Tübingen **1**: 636–685. 1881–1885.

[4] **Palladin, W.,** Sur le rôle des hydrates de carbone dans la resistance à l'asphyxie chez les plantes supérieures. Rev. gén. bot. **6**: 201–209. 1894.

[5] **Palladin** and **Kostytschew,** 1906, 1907. [See note 3, p. 215.]

According to Kostychev's[1] experiments, Psalliota (Agaricus, the ordinary cultivated mushroom) forms considerable amounts of carbon dioxide but no trace of alcohol, when grown under anaerobic conditions. This mushroom was found to contain no sugar at all.[2] The same writer found that *Aspergillus niger*,[3] grown anaerobically in a medium without carbohydrates, produces much carbon dioxide. Whatever may be the decomposition products arising in this case, it is clear that anaerobic respiration is not *always* the same thing as alcoholic fermentation. Although it was long supposed[4] that plants containing mannite eliminate not only carbon dioxide but also molecular hydrogen, when deprived of oxygen, Kostychev[5] was unable to detect any production of hydrogen by such plants.

When plants are transferred to aerobic conditions after a prolonged period without oxygen, an accelerated production of carbon dioxide is sometimes observed.[6] This may be explained by supposing that unoxidized decomposition products of anaerobic respiration are oxidized as soon as oxygen becomes again available.

Both lower and higher plants consume more nutritive materials during anaerobic than during aerobic respiration. The processes of oxidation (respiration) are thus more efficient from the standpoint of the organism than are those of reduction (fermentation).[7] Anaerobic respiration, like aerobic, is influenced by many kinds of conditions, some of which accelerate while others retard the production of the carbon dioxide.[8]

§9. Respiration Chromogens.[k]—Very widespread in plants are a group of substances called by Palladin[9] *respiration chromogens*. To obtain them, an

[1] **Kostytschew, S.,** Ueber anaërobe Athmung ohne Alkoholbildung. Ber. Deutsch. Bot. Ges. **25**: 188–191. 1908. **Idem,** Zweite Mitteilung über anaërobe Atmung ohne Alkoholbildung. *Ibid.* **26a**: 167–177. 1908.

[2] **Kostytschew, S.,** Ein eigentümlicher Typus der Pflanzenatmung. Zeitsch. physiol. Chem. **65**: 350–382. 1910.

[3] **Kostychev, S.,** Untersuchungen über die anaërobe Athmung der Pflanzen. [Abstract in German, p. 155–162. Text in Russian.] Scripta Botanica Hort. Univ. Imp. St. Petersburg **25**: 1–162. 1907.

[4] **Müntz, A.,** Recherches sur les fonctions des champignons. Ann. chim. et phys. *V*, **3**: 56–92. 1876. **Luca, Sebastiano de,** Recherches chimiques tendant à démonstrer la production de l'alcool dans les feuilles, les fleurs et les fruits de certaines plantes. Ann. sci. nat. Bot. *VI*, **6**: 286–302. 1878.

[5] **Kostytschew, S,.** Zur Frage über die Wasserstoffausscheidung bei der Atmung der Samenpflanzen. Ber. Deutsch. Bot. Ges. **24**: 436–441. 1906. **Idem,** Zur Frage der Wasserstoffbildung bei der Atmung der Pilze. *Ibid.* **25**: 178–188. 1907.

[6] **Palladin, W.,** Ueber normale und intramolekulare Atmung der einzelligen Alge Chlorothecium saccharophilum. Centralbl. Bakt. *II*, **11**: 146–153. 1904.

[7] **Palladin, V. I. [W.],** [Title and text in Russian.] Bull. Soc. Imp. Nat. Moscow **62**^{II}: 44–126. 1886. **Palladin, W.,** Bedeutung des Sauerstoffs für die Pflanzen. (Extract from the Russian paper just cited.) *Ibid.* **62**^{II}: 127–133. 1886.

[8] **Smirnoff,** 1903. [See note 6, p. 213.]. **Kostytschew,** 1902. [See note 3, p. 87.] **Morkowin. N.,** Ueber den Einfluss der Reizwirkungen auf die intramolekulare Atmung der Pflanzen. Ber. Deutsch. Bot. Ges. **21**: 72–80. 1903.

[9] **Palladin, V. I. [W.],** Sur la repartition et la formation des chromogênes respiratoires dans les plantes. Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, **2**: 977–990. 1908. [This work is also reported in the next two references.] **Palladin, W.,** Die Verbreitung der Atmungschromogene bei den Pflanzen. Ber. Deutsch. Bot. Ges. **26a**: 378–389. 1908. **Idem,** Ueber die Bildung der Atmungschromogene in den Pflanzen. *Ibid.* **26a**: 389–394. 1908. **Palladin, V. I. [W.],** Sur le prochromogénes des chromogénes respiratoires des plantes. [Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, **3**: 371–376. 1909. [This is also reported in the next reference.] **Palladin, W.,** Ueber Prochromogene der Pflanzlichen Atmungschromogene. Ber. Deutsch. Bot. Ges. **27**: 101–106. 1909. **Palladin, V. I. [W.],** Contributions à la physiologie des lipoides. [Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, **4**: 785–795. 1910. **Palladin, W.,** Synergin, das Prochromogen des Atmungsfermente der Weizenkeime. Biochem. Zeitsch. **27**: 442–449. 1910.

[k] Sections 9 and 10 are translated from the 7th Russian edition; they differ from the corresponding sections (10 and 9) of the German edition.—*Ed.*

extract of the plant tissue is prepared with boiling water and filtered. The addition of peroxidase and hydrogen peroxide to the filtrate thus obtained produces a red (rarely lilac or violet) color, due to the respiration pigment formed by oxidation of the chromogen, and this rapidly changes with further oxidation, to a dark violet or black.

Respiration chromogens appear to exist in plant tissues mainly in the form of pro-chromogens, which may be glucosides. To obtain the pro-chromogen of wheat embryos, the material is first extracted with alcohol and the pro-chromogen is precipitated from the extract, by acetone. It is soluble in water and is decomposed by emulsin, with the production of the chromogen. The latter is oxidized by peroxidase, without hydrogen peroxide, into the red respiration pigment. The experiments of Combes[1] showed that the transformation of the chromogen into the pigment is accompanied by increased respiratory activity. An alkaline solution of chromogen absorbs oxygen very actively.[2] Some of the natural plant dyes are obtained by the complete oxidation of chromogens.[3]

The chromogens appear to belong in the same class with ortho-dioxy-benzene.[4] Urushiol, the chromogen of Japanese lacquer (from *Rhus vernicifera*, etc.), has the formula $C_{20}H_{30}O_2$ and its structure is that of o-dioxy-benzene with a large, unsaturated side-chain.

By forming water, the respiration chromogens remove the hydrogen produced by the respiration process. If the pigment be represented by the letter R, this reaction is shown by the equation: R (pigment) + H_2 (hydrogen) = R-H_2 (chromogen). By the action of oxidase, the chromogen, as it is produced, absorbs oxygen from the air and forms water and the pigment, as according to the equation: R-H_2 (chromogen) + O (oxygen) = H_2O (water) + R (pigment). Thus the respiration pigments may be regarded as *acceptors* of hydrogen (see page 207.)

§10. **Respiratory Enzymes.**[5]—Recent studies agree in indicating that plant respiration is the summation of a number of fermentation or enzymatic processes. If plants are killed without destroying their enzymes, the production of carbon dioxide and the absorption of oxygen still continue, but in such cases only the primary, anaerobic phase of the process (corresponding to alcoholic fermentation) is present. In some kinds of plants thus killed, in spite of the fact that they are plentifully supplied with peroxidase, the secondary, direct-

[1] **Combes, Raoul,** Les échanges gazeux des feuilles pendant la formation et la destruction des pigments anthocyaniques. Rev. gén. bot. 22: 177–212. 1910.

[2] **Rupe, Hans,** Die Chemie der natürlichen Farbstoffe. **Braunschweig,** 1900, 1909. 2 v. [This statement and citation are omitted in the 7th Russian Edition.—*Ed.*]

[3] **Palladin, V. I.** [W.], and **Tolstaia, Z. N.,** Sur l'absorption de l'oxygène par les chromogènes respiratoires des plantes. [Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, 7: 93–108. 1913. [Also reported in the following reference.] **Palladin, W.,** and **Tolstaja, Z.,** Ueber die Sauerstoffabsorption durch die Atmungschromogene der Pflanzen. Biochem. Zeitsch. 49: 381–397. 1913.

[4] **Majima, R.,** and **S. Chō,** Ueber einen Hauptbestandteil des japanischen Lackes. (Vorläufige Mitteilung.) Ber. Deutsch. Chem. Ges. 40^{IV}: 4390–4393. 1907. **Majima, Riko,** Ueber den Hauptbestandteil des Japanlacks. (I. Mitteilung.) Ueber Urushiol und Urushiol-dimethyläther. *Ibid.* 42^{I}: 1418–1423. 1909. **Idem,** Ueber den Hauptbestandteil des Japanlacks. (II. Mitteilung.) Die Oxydation des Urushiol-dimethyläthers mit Ozon. (I. Mitteilung.) *Ibid.* 42^{III}: 3664–3673. 1909. **Idem,** Ueber den Hauptbestandteil des Japanlacks. (III. Mitteilung.) Die katalytische Reduktion von Urushiol. *Ibid.* 45^{II}: 2727–2730. 1912.

[5] **Palladin,** 1909. [See note 3, p. 204.]

oxidation phase is entirely absent; in other kinds of plants direct oxidation occurs, but quite differently from its occurrence in the living organism.

In an atmosphere free from oxygen the anaerobic phase of aespiration, in plants killed without injury to the enzymes, is accompanied by the production of both carbon dioxide and ethyl alcohol, but in most plants the production of alcohol is the less vigorous of the two processes. Under such conditions the formation of carbon dioxide ceases after a time, and if oxygen is then admitted to the tissues this formation may begin again or not, according to the kind of plant employed. In some forms (*e.g.*, wheat embryos) no further production of carbon dioxide takes place. In other forms the killed plants (with their enzymes still intact) give off carbon dioxide even more vigorously in the presence of oxygen than they did in its absence. In this latter case, however, the carbon dioxide produced after the admission of oxygen is not to be considered as the product of direct oxidation. For example, Palladin and Kostychev[1] found that germinating peas, killed without injury to the enzymes, developed considerably larger amounts of carbon dioxide when air was admitted than they had done in the absence of oxygen. Alcohol formation was likewise increased, however, so that the acceleration of carbon dioxide formation cannot be regarded as the direct result of oxidation. In such cases Ivanov[2] supposes that the oxygen is supplied by the activity of the enzyme zymase.

Another example may be presented. Etiolated bean leaves that had been killed by freezing were deprived of oxygen until carbon dioxide ceased to be given off, after which air was admitted, when the elimination of carbon dioxide was resumed and the leaves became black, as a result of the oxidation of the chromogen. Although the renewed production of carbon dioxide was not here accompanied by alcohol formation, still we must refrain from supposing that it was the direct result of oxidation, as will become clear from the following considerations.

Anaerobic decomposition is accompanied not only by the evolution of carbon dioxide but also by the production of hydrogen. In some plants this hydrogen disappears in the reduction (to form alcohol) of intermediate products of the decomposition, but in most plants little alcohol is formed. In the latter case the hydrogen must unite either with the respiration pigment or with some other hydrogen acceptor, and when all acceptors of hydrogen become satisfied (having taken up all the hydrogen they can) there should be no further decomposition (and, consequently, no more evolution of carbon dioxide). When the cells are exposed to the air the acceptors of hydrogen oxidize their hydrogen to water, however, and thus become able to absorb still more hydrogen. Therefore, exposure to air results first in the regeneration of the hydrogen acceptors, which is accompanied by a renewal of hydrogen absorption and a consequent renewal of anaerobic decomposition, the latter being, of course, accompanied by the giving-off of carbon dioxide, just as occurred at first. It is thus seen how this

[1] Palladin and Kostytschew, 1906. [See note 3, p. 215.]

[2] Iwanoff, Leonid, Ueber die Sogenannte Atmung der zerriebenen Samen. Ber. Deutsch. Bot. Ges. 29: 563–570. 1911.

carbon dioxide is the result of anaerobic respiration rather than of oxidation by free oxygen (compare page 221). When oxygen is first admitted to tissues in which the hydrogen acceptors have already been satisfied, the renewed evolution of carbon dioxide is very vigorous.

As has been stated (pages 200, 208), in the presence of methylene blue and a catalyzer, formic acid decomposes into carbon dioxide and hydrogen, so long as absence of oxygen prevents the regeneration of the methylene blue from the leuco-compound formed from the dye by union with hydrogen; $HCOOH + M = CO_2 + M\text{-}H_2$, where M represents methylene blue and $M\text{-}H_2$ represents the leuco-compound. Access of oxygen allows the removal of the extra hydrogen from the leuco-compound and regenerates the methylene blue ($M\text{-}H_2 + O = M + H_2O$), thus rendering it again able to absorb hydrogen. Consequently, the evolution of carbon dioxide begins anew and it appears, superficially, as though this were the result of the oxidation of the carbon of the formic acid by atmospheric oxygen. Here the methylene blue behaves as an acceptor of hydrogen, as such acceptors are supposed to act in plant respiration.

Bach and Batelli,[1] and also Palladin,[2] regard all the carbon dioxide eliminated in respiration as the product of anaerobic fermentation. Palladin thinks water enters into this decomposition reaction; thus, $C_6H_{12}O_6$ (glucose) $+ 6\ H_2O = 6\ CO_2 + 12\ H_2$. Since much hydrogen should result from this sort of reaction and since hydrogen is never actually given off by higher plants, it follows that the differing capacities of different kinds of plants for the anaerobic evolution of carbon dioxide depend upon the various powers of the plants to carry out reductions that result in alcohol, and upon the differing amounts of hydrogen acceptors present.

In living plants, the hydrogen produced by anaerobic decompositions is taken up by the respiration pigments, forming the corresponding chromogens. From these it is subsequently removed and oxidized to form water, through the action of oxidase. The reactions are shown by the two following equations, where R represents the pigment and $R\text{-}H_2$ the chromogen. (1) $12\ H_2 + 12\ R = 12\ R\text{-}H_2$. (2) $12\ R\text{-}H_2 + 6\ O_2 = 12\ H_2O + 12\ R$. It thus appears that the respiration enzymes are water-producing enzymes, carrying out the same reactions in the living plant as they do *in vitro*. Thus oxidase (or peroxidase together with hydrogen peroxide) oxidizes colorless hydroquinone (chromogen) to form red quinone (pigment) and water, according to the equation:

$$\underset{\text{Hydroquinone}}{C_6H_6O_2} + O = \underset{\text{Quinone}}{C_6H_4O_2} + H_2O.$$

In plants that have been killed without destroying their enzymes the controls that govern the various activities during life are greatly disturbed, and the respiration pigments in such tissues remove not only the hydrogen that they normally take up (this being then oxidized to form water), but also the hydrogen simultaneously being produced by, and taking part in, the anaerobic processes. Consequently, such killed tissues that are rich in chromogens give off more

[1] **Bach, A.,** and **Battelli, F.,** Dégradation des hydrates de carbone dans l'organisme animal. Compt. rend. Paris **136**: 1351–1353. 1903.

[2] **Palladin,** 1912 (*1*, *2*). [See note 3, p. 207.]

carbon dioxide in the air when they have been previously kept for a time in an atmosphere free from oxygen. This fact has been mentioned before, but the following example will make it clearer.

Of two portions of frozen, etiolated bean leaves, one portion was exposed to the air for sixty-three hours and the other was first exposed for twenty-three hours to an atmosphere of hydrogen, and then to the air for forty hours. The first portion (in air for sixty-three hours) gave off 286 mg. of carbon dioxide. The second portion gave off 183 mg. of carbon dioxide during its twenty-three hours in hydrogen and 245 mg. during the succeeding forty hours in air, or 428 mg. during the entire sixty-three hours. During the whole period the second portion gave off 50 per cent. more carbon dioxide than did the other. In the first portion (in air all the time) the respiration pigments removed a part of the active hydrogen produced by the first stage of anaerobic respiration, and therefore exerted the same retarding influence upon the process as was evident in the experiments of Palladin and Lvov (see page 207), in which the chromogen of the beet or methylene blue retarded alcoholic fermentation. In considering plant respiration it is thus necessary to distinguish between the hydrogen that is normally taken up by the hydrogen acceptor and is then oxidized to form water, and the other, more active, hydrogen that is simultaneously being produced by the anaerobic reactions under the influence of reductase, as in the formation of alcohol by reduction. This latter hydrogen is necessary for the continuation of the anaerobic process.

As has been stated previously (pages 213), the respiration process is accelerated by wounding. Krasnosselskaia[1] has shown that this acceleration is concomitant with an increase in the amount of anaerobic enzymes (such as zymase, perhaps) and also with an increase in the amount of peroxidase present in the tissues. Four equal portions of leek bulbs that had been wounded and allowed to remain alive for one, four, seven and fifteen days, respectively, were finally frozen and treated with pyrogallol and hydrogen peroxide. The four portions produced 25.2, 74.8, 149.6, and 200.4 mg. of carbon dioxide, respectively, which indicates the progressive increase in the amount of respiration enzymes in the wounded bulbs.

Poisons also accelerate plant respiration (see page 213), but without increasing the respiration enzymes.[2] Two similar lots of etiolated stems of *Vicia faba* were kept in darkness for some time, with their cut ends in sugar solution. One lot was then treated with quinin, and produced 21.4 mg. of carbon dioxide in two hours, while the lot without the alkaloid formed only 11.3 mg. Both lots were then killed by freezing. After thawing, the one with quinin produced 37.2 mg. of cabon dioxide in twenty-five hours, while the other formed 37.6 mg. A very marked acceleration in the evolution of carbon dioxide is seen to have been

[1] **Krasnosselsky, T.**, Bildung der Atmungsenzyme in verletzten Pflanzen. Ber. Deutsch. Bot. Ges. 23: 142–155. 1905. **Idem,** Bildung der Atmungsenzyme in verletzten Zwiebeln von *Allium cepa*. *Ibid.* 24: 134–141. 1906.

[2] **Palladin, V. I. [W.]**, Respiration des plantes comme somme des proces de fermentation. [Russian. Mém. Acad. Imp. Sci. St.-Pétersbourg *VIII*, 20[5]: 1–64. 1907. **Idem,** sur l'action des poisons sur la respiration des plantes. [Russian.] Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, 4: 401–421. 1910. [This is also reported in the reference given in note 3, p. 213.]

produced by quinin treatment when the tissues were still alive, but the alkaloid exerted no accelerating influence in the case of the frozen and thawed tissues, which were dead but still contained their enzymes.

Respiration in living plants is thus accelerated, not only by certain substances that are necessary for life (such as co-enzymes), but also by unnecessary and generally injurious substances (poisons, in the usual sense). Both kinds of substances produce the same result, namely an acceleration of respiratory activity, but the chemical responses within the cells are quite different in the two cases. In one case we have to do with phenomena of nutrition and in the other case with those of poisoning. In living plants this difference is not apparent, but Ivanov[1] has clearly demonstrated it with plants that were killed without destroying their enzymes. Phosphates, which belong to the class of necessary accelerators, produce a marked influence, both upon living plants and upon those that have been killed but that still retain their enzymes.

§11. **Materials Consumed in Respiration.**—Notwithstanding the fact that respiration in plants is accompanied by a decrease in carbohydrates and fats, which are non-nitrogenous, it was generally supposed until quite recently that such nitrogen-free compounds were not directly consumed in this process and that atmospheric oxygen acted directly to oxidize only proteins. The nitrogenous residues left as products of protein decomposition were supposed to combine with carbohydrates, thus regenerating the proteins. According to this conception, as long as the supply of reserve carbohydrates is not exhausted the amount of protein material in the organism remains unchanged, while the non-nitrogenous reserve gradually diminishes; but as soon as the reserve of carbohydrates has been exhausted then decomposition of proteins becomes apparent and the nitrogenous products of this decomposition begin to accumulate. Evidence in favor of the idea that protein is directly oxidized in respiration was found in the fact that the respiration process is especially active in young, growing tissues, rich in protein. This conception has proved to be untenable, however.

The protein of the organism does not remain constant in amount as long as carbohydrates are available; in the germination of seeds, for example, the decomposition of proteins proceeds most rapidly in the earliest stages of germination, when the seeds are still very rich in carbohydrates. With decreasing carbohydrate content protein decomposition becomes less vigorous and finally may even cease altogether. To give an illustration of this, 100 g. of wheat seeds contained 0.0668 g. of protein nitrogen, and etiolated seedlings six days old, from a similar lot of seeds, contained only 0.0554 g., so that 0.0114 g. of protein nitrogen had been lost during germination. When the seedlings were fourteen days old their content in protein nitrogen was 0.0549 g., so that only 0.0005 g. had been lost during the last eight days. The progress of protein decomposition in dark-grown wheat seedlings has been graphically shown in Fig. 88.

Carbohydrates are necessary for aerobic respiration, even in the presence of

[1] **Ivanov, N. N.,** Action des agents stimulants utiles et nuisibles sur la respiration des plantes. [Russian. Bull. Acad. Imp. Sci. St.-Pétersbourg *VI*, 4: 571–581. 1910. This is also reported in the second reference given in note 1, p. 214.]

an excess of proteins. Etiolated bean leaves, which are rich in protein but contain only a little carbohydrate, produce carbon dioxide at an exceedingly low rate; Palladin[1] found that 100 g. of such leaves, at room temperature, gave off carbon dioxide for three successive hours at the rates of 102.8, 95.9, and 70.2 mg., respectively, with an average rate of 89.6 mg. per hour. The same leaves were floated upon cane-sugar solution in darkness for two days, by which treatment their carbohydrate content was markedly increased without serious alteration of their protein content, and they then gave off carbon dioxide for four successive hours at the rates of 152.6, 147.5, 146.8, and 144.5 mg., respectively, with an average rate of 147.8 mg. per hour.

If etiolated bean leaves are kept upon cane-sugar solution longer than two days their carbohydrate content continues to increase, but this further increase in carbohydrates is without influence upon the rate of elimination of carbon dioxide. After forty hours upon cane-sugar solution 100 g. of these leaves produced 144.5 mg. of carbon dioxide in one hour. After forty-two hours longer upon the sugar solution they gave off 144.1 mg. of carbon dioxide in an hour. The longer period upon sugar solution, although resulting in higher carbohydrate content, did not produce any alteration in the respiration rate; the protein content of the leaves remained unchanged and the supply of carbohydrates was adequate in both cases. This experiment shows that there exists no constant relation between the rate of evolution of carbon dioxide and the supply of carbohydrates. During the shorter period upon sugar solution these leaves had absorbed enough sugar so that the sugar content of the tissues was adequate for the maximum respiration rate with the given amount of proteins, and still further addition of sugar was without influence upon the rate of elimination of carbon dioxide.

An excess of carbohydrates is to the living cell what a coal supply is to a manufacturing establishment; as long as there is sufficient coal on hand to operate the machinery at maximum speed, the amount of the coal supply determines only *how long* the factory can be kept in operation, and is without influence upon the daily rate of production. The daily output from such an establishment, so long as enough coal is available to operate the machines at their maximum speed, is dependent only upon the capacity of the machines themselves. Similarly, only the *duration* of the respiration process in a cell is dependent upon the supply of carbohydrates present, providing only that the supply is adequate for the maximum rate, and this maximum rate depends upon the capacity of the living protoplasm to carry on the respiratory process. Other conditions remaining the same, this capacity depends upon the amount of protoplasm present in the cell. Regarding the cell as a factory, carbohydrates are the coal and the protoplasm is the machinery. Only upon the amount of protoplasm present does the rate of the life-processes thus depend, assuming the supply of carbohydrates, water, etc., to be adequate and the temperature, etc., to be optimum.

Carbohydrates are not directly acted upon by the protoplasm, but their de-

[1] **Palladin, W.**, Recherches sur la respiration des feuilles vertes et des feuilles étiolées. Rev. gén. bot. 5: 449–473. 1893.

composition is brought about by the action of specific enzymes, the amount of which depends upon the amount of protoplasm present. As has been noted (page 157), not all proteins are to be regarded as constituents of the living protoplasm; the plant cell contains larger or smaller amounts of non-protoplasmic proteins, and the question arises whether the respiration rate is a function of the total protein content or of the protoplasmic proteins only. During germination in darkness the total protein content is lowered while the rate of carbon dioxide production gradually rises (see pages 180, 227), so that seedlings with little protein, in the later stages of germination, respire more vigorously than do seedlings with more protein, in earlier stages. During this process of germination in darkness, however, it is only the non-protoplasmic or reserve proteins that decrease; the proteins that are indigestible in gastric juice, which are just the ones that are to be considered as part of the protoplasm, increase during germination (see Fig. 88, p. 181). Palladin[1] carried out parallel series of determinations of the amounts of carbon dioxide given off by, and of indigestible proteins[l] present in, wheat seedlings during germination in darkness. These determinations showed that, in the intermediate stages of germination, with adequate supply of carbohydrates, the rate of elimination of carbon dioxide is proportional to the amount of indigestible protein present in the plantlet.[2] In later stages of germination, as has been said, the respiration rate decreases, on account of the diminishing supply of carbohydrates, but the indigestible proteins still continue to increase in amount.

With the same temperature and with adequate carbohydrate supply, equal amounts of carbon dioxide are produced per unit of time, for a given amount of indigestible proteins. In the case of wheat germinating at a temperature of from 20 to 21°C., the ratio of the hourly rate of carbon dioxide production to the amount of nitrogen in the indigestible proteins of the seedling $\left(\frac{CO_2}{N}\right)$ had the following values, at successive stages of germination; seedlings four days old, 1.06; six days old, 10.5; seven days old, 1.18; nine days old, 1.15. It thus appears that, with a plentiful supply of carbohydrates, the respiratory rate depends upon the amount of nuclein materials (taken to be proportional to the amount of proteins indigestible in gastric juice) that are present in the seedling.

This conclusion is also supported by the observation of Burlakov,[3] that embryos respire much more actively in proportion to their weight than do entire seeds. One hundred grams of wheat seeds, after soaking in water forty-eight hours, gave off carbon dioxide at the rate of 15.2 mg. per hour, at a temperature of from 20 to 22°C. The same weight of separate embryos, after soaking twenty-four hours, produced 241.8 mg. per hour. The respiration of the em-

[1] **Palladin,** 1896. [See note 6, p. 180.]

[2] Although the amount of living protoplasm in the plant may thus be approximated in terms of the amount of indigestible protein, the method is confessedly not precise, as more recent studies show. It was the best available for these experiments, however.

[3] **Burlakov, G. G.,** Sur la question de la respiration du germe de froment. [Russian.] Trav. Soc. Imp. Nat. Univ. Kharkov **31**: *V–XV*. 1897. [Pagination in Roman numerals.]

[l] The term indigestible, as used in the text, refers to those proteins that are found to be undigested by gastric juice.—*Ed.*

bryos, which were much richer in nuclein substances, was thus seventeen times as vigorous as that of the entire seeds.

Respiration in seed-plants occurs, in general, by the destruction of carbohydrates. Its dependence upon proteins is due (1) to the fact that carbohydrates may be formed, under certain conditions, from proteins, and (2) to the fact that respiratory enzymes are formed by the protoplasm; on the amount of protoplasm depends the amount of these enzymes, and, consequently, the rate of respiratory activity.

§12. **Special Cases of Respiration in Lower Plants.**—In many lower forms of plant life carbohydrates are not the substances that are decomposed in respiration. Among these forms occur not only various types of fermentation, as has been pointed out, but also various types of aerobic respiration. The respiration process in acetic-acid bacteria, for example, is just an oxidation of ethyl alcohol to acetic acid, according to the following equation:

$$\underset{\text{Ethyl Alcohol}}{CH_3CH_2OH} + \underset{\text{Oxygen}}{O_2} = \underset{\text{Acetic Acid}}{CH_3COOH} + \underset{\text{Water}}{H_2O}.$$

This process is really not a fermentation at all, in the restricted sense of this term; it is to be regarded as a special kind of aerobic respiration. The true fermentations (anaerobic respiration) are characterized by the decomposition of complex compounds into simpler ones, while oxidations are characteristic of aerobic respiration. As long as alcohol is present, the end product of the respiration of these organisms is acetic acid, but as soon as the supply of alcohol has been completely consumed they begin to oxidize acetic acid into carbon dioxide and water.

Pasteur was the first to recognize acetic acid fermentation as a vital process, and he thought that the bacteria controlling it were of the single species, *Mycoderma aceti.* Hansen[1] showed later that the bacterial membranes (mother) arising during this process consist mainly of three different species of bacteria, *Bacterium aceti*, *Bacterium pasteurianum* and *Bacterium kuetzingianum.* These three forms are briefly described below.

Bacterium aceti, when grown on beer at room temperature, forms (in twenty-four hours) a smooth slimy skin, which consists of chains of rod-like cells (Fig. 94). These cells are colored yellow by iodine. With a temperature of from 40 to 45°C. the rod-like cells form long, thin filaments.

Bacterium pasteurianum, grown on beer, forms a dry superficial skin, which is usually wrinkled. This consists also of chains of rod-like cells (Fig. 95), but the latter are larger than in the form just described. The slimy layer surrounding the cells of a newly-formed skin is colored blue by iodine.

Bacterium kuetzingianum forms, on beer at 34°C., a dry surface skin which grows upward at the edges, on the walls of the culture-vessel. The skin consists of rod-like cells but these do not occur in rows or chains but are generally single or joined in pairs. The slime about the cells is colored blue by iodine, as in the last form.

[1] **Hansen, Emil Christian,** Recherches sur les bactéries acétifiantes. Compt. rend. trav. Lab. Carlsberg, Kjöbenhavn 3^{III}: 182–216. 1894. [Rev. by Fr. **Lahar** in Bot. Zeitg. 52^{II}: 337–342. 1894.]

Besides the three bacteria just described other bacteria are also employed in the manufacture of vinegar. *Bacterium xylinum* is commonly used in England.

The oxidation of alcohol to acetic acid is carried on in the cells of these bacteria by a specific intracellular enzyme. Buchner and Gaunt[1] obtained acetone preparations of acetic acid bacteria, which, like Buchner's "zymin" (see page 167), possessed keeping qualities, and had the power causing the oxidation of alcohol to acetic acid.

Another special kind of aerobic respiration, similar to that of the acetic acid bacteria just considered, is that of the sorbose bacteria,[2] which merely oxidize sorbite to sorbose. The following equation represents the reaction:

$$\underset{\text{Sorbite}}{2\ C_6H_{14}O_6} + \underset{\text{Oxygen}}{O_2} = \underset{\text{Sorbose}}{2\ C_6H_{12}O_6} + \underset{\text{Water}}{2\ H_2O}.$$

Still other alcohols are oxidized by microörganisms, producing the corresponding aldehydes and ketones. Such a physiological oxidation process furnishes

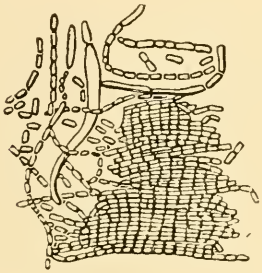


FIG. 94.—*Bacterium aceti*, skin formed at the surface of beer. (Highly magnified.)

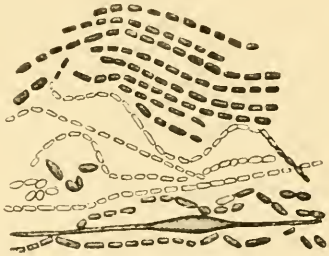


FIG. 95.—*Bacterium pasteurianum*, cells from skin formed at the surface of beer. (Highly magnified.)

the best method for obtaining dihydroxyacetone from glycerine, the reaction being as represented below.

$$\underset{\text{Glycerine}}{2\ CH_2OH\text{-}CHOH\text{-}CH_2OH} + \underset{\text{Oxygen}}{O_2} = \underset{\text{Dihydroxyacetone}}{2\ CH_2OH\text{-}CO\text{-}CH_2OH} + \underset{\text{Water}}{2\ H_2O}.$$

The nutrition of bacteria by mineral substances, which has been previously considered (see page 47), also really represents special cases of aerobic respiration. One form of bacteria oxidizes hydrogen sulphide, another oxidizes ammonia, a third oxidizes hydrogen, etc. The cosmic importance of these special types of physiological oxidation is very great, for it is through these processes that the natural circulation of sulphur, nitrogen, and hydrogen is largely brought about. The total amounts of the various chemical elements available for life-processes upon our planet remain practically constant, but the various compounds are always decomposing and being reformed, so that the elements are forever in a state of circulation, and bacteria play a very important rôle in this great process.

[1] **Buchner, Eduard,** and **Gaunt, Rufus,** Ueber die Essiggährung. Liebig's Ann. Chem. u. Pharm. 349: 140–184. 1906.

[2] **Bertrand, G.,** Étude biochimique de la bactérie du sorbose. Ann. chim. et phys. *VIII*, 3: 181–288. 1904.

§13. **Circulation of Energy in Nature.**—The circulation of energy is quite different from that of matter. The available supply of energy upon the earth is inadequate for a long continuation of plant and animal life, which would soon cease were it not for the continuous influx of energy from the sun. From the law of the conservation of energy it is clear that the solar energy stored up in potential form by the photosynthetic process in green plants must be completely liberated by the reverse process (the formation of carbon dioxide and water), as this occurs in combustion or in plant and animal respiration. The carbon dioxide thus produced can, of course, enter again into organic compounds, but that portion of the energy liberated by respiration and fermentation that takes the form of heat is almost entirely lost from the organism and does not again become available for organic synthesis; it becomes dissipated into space and is gone forever from the earth. Thus vital activity upon our planet is directly dependent upon the sun, from which new supplies of energy must continually come if life is to be long continued.

This process of energy dissipation may be illustrated somewhat as follows. If a small beaker of hot water is poured into a large tank of cold water, the cold water is warmed but little; supposing the original temperatures to be 95° and 5°, respectively, the temperature of the tank may perhaps rise to 6° when the hot water is added. At first the heat energy is concentrated, or intensive, in the beaker; later it is dissipated, or extensive, in the tank. The coefficient of energy dissipation, that fraction of the original energy that can no more be converted into mechanical work, is termed entropy, and entropy always tends toward a maximum. In this process of the dissipation of the intensive energy of our solar system, plants play a direct rôle.

Summary

1. **General Discussion of Fermentation and Respiration.**—All changes and movements of materials involve changes with respect to *energy*, and the material transformations and movements that occur within the living plant are not exceptions to this principle. Some of the processes of living tissues result in the setting free of energy, while others of these processes result in energy fixation or accumulation. The photosynthesis of carbohydrates from carbon dioxide and water, in chlorophyll-bearing tissue, results in the transformation of radiant energy (sunlight) into the stored potential energy of the combustible compounds that are formed; this is therefore an energy-fixation process, and the fixed energy remains in the plant. Transpiration is a process by which kinetic energy (light, heat) is transformed into the potential form, as the latent heat of water vapor; it is an energy-fixation process, but the fixed energy leaves the plant. Whenever a carbohydrate is oxidized in the living tissue, energy is set free, just as when wood is burned. Some of the energy resulting from this oxidation escapes from the plant, but much of it is consumed in other chemical processes and is retained for a longer or shorter period. Most of the chemical transformations that occur in the living protoplasm take place because of, and at the expense of, energy that is set free by oxidations. The processes that set energy free in this way, in the living organism, are grouped together as the process of respiration.

It appears that the oxygen consumed in the primary steps of respiration is generally not free oxygen (O_2); it is derived from compounds that contain other elements (especi-

ally carbon and hydrogen) as well as oxygen. The oxygen of one part of a complex molecule may oxidize even another part of the same molecule. These primary steps of respiration are called *intramolecular respiration*, *anaerobic respiration*, or *fermentation*. They proceed under the influence of fermentation enzymes and they result in incomplete oxidation, some or all of the new substances produced being only partially oxidized; but considerable amounts of energy are set free by these fermentation processes. The partially oxidized substances thus formed may accumulate in, and escape from, the organism without further alteration, or their oxidation may be completed by the action of oxidizing enzymes in the presence of an adequate supply of free oxygen, the ultimate products being then all completely oxidized. In the presence of free oxygen, the products of the primary fermentation processes may be different, or complete oxidation may occur before they are formed. The final steps of the respiration process are called *normal respiration* or *aerobic respiration*. In aerobic respiration, free oxygen is absorbed, and completely oxidized substances are produced, with the setting free of much more energy than was previously made available by the anaerobic processes. Intramolecular or anaerobic respiration, or fermentation, occurs in every living cell; but there are many kinds of cells in which aerobic respiration does not occur, either because the cells lack the necessary oxidizing enzymes or else because free oxygen is not supplied. Some microörganisms are unable to live at all in the presence of free oxygen (obligate anaerobes), and their respiration is consequently limited to the primary fermentation processes; these forms give off products, some or all of which are incompletely oxidized. Organisms that thrive in the presence of free oxygen may give off some incompletely oxidized substances even with a good supply of oxygen—as if these substances diffused out of the cells before the completion of oxidation. With an inadequate supply of oxygen, fermentation products generally become conspicuous, as in the roots of ordinary plants in poorly aerated soil (see Chapter V, Section 5).

The chemistry of respiration may be pictured superficially by means of the following equations, which are to be considered simply as illustrations of the kinds of processes that are apparently involved.

I. *Fermentation or anaerobic processes*:—

1. Oxygen from the same molecule.

$$\underset{\text{Glucose Sugar}}{C_6H_{12}O_6} = \underset{\text{(oxidation incomplete)}}{\text{Ethyl Alcohol } 2\,C_2H_5OH} + \underset{\text{(oxidation complete)}}{\text{Carbon Dioxide } 2\,CO_2}$$

2. Oxygen from another compound.

$$\underset{\text{Glucose Sugar}}{C_6H_{12}O_6} + \underset{\text{Water}}{6\,H_2O} = \underset{\text{(oxidation incomplete)}}{\text{Hydrogen } 12\,H_2} + \underset{\text{(oxidation complete)}}{\text{Carbon Dioxide } 6\,CO_2}$$

II. *Aerobic processes (with free oxygen)*:—

$$1.\ \underset{\text{Glucose Sugar}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\,O_2} = \underset{\text{Water}}{6\,H_2O} + \underset{\text{Carbon Dioxide}}{6\,CO_2}$$

$$2.\ \underset{\text{Ethyl Alcohol}}{C_2H_5OH} + \underset{\text{Oxygen}}{3\,O_2} = \underset{\text{Water}}{3\,H_2O} + \underset{\text{Carbon Dioxide}}{2\,CO_2}$$

$$3.\ \underset{\text{Hydrogen}}{2\,H_2} + \underset{\text{Oxygen}}{O_2} = \underset{\text{Water}}{2\,H_2O}$$

When a certain amount of any substance is transformed by fermentation, the quantity of energy set free is much less than when aerobic respiration occurs and

oxidation is complete. The fermentation of a gram-molecule of glucose (forming 2 g.-molecules of ethyl alcohol and 2 g.-molecules of carbon dioxide) should give 57 kg.-cal. of heat, while the complete oxidation (to water and carbon dioxide) of the alcohol thus produced (2 g.-molecules) should give 652 kg.-cal. in addition. The complete oxidation of a g.-molecule of glucose should consequently give 57 + 652, or 709, kg.-cal. To set free a given amount of free energy, more than twelve times as much glucose must be used in fermentation as would be required in aerobic respiration.

2. Alcoholic Fermentation by Yeast.—The Saccharomycetes absorb sugar from the medium and derive free energy from the decomposition of this sugar by means of the respiration enzyme zymase. The main products of this *alcoholic fermentation* are ethyl alcohol and carbon dioxide, which diffuse from the cells into the medium. Some nitrogenous material and mineral salts, as well as sugar, are necessary for the growth of yeasts, just as for that of other plant forms.

It appears that this fermentation process proceeds by two stages, employing an inorganic phosphate (such as K_2HPO_4) as a co-enzyme, along with zymase. In the first stage carbon dioxide, ethyl alcohol, water, and a hexose phosphate are produced. In the second stage the hexose phosphate is decomposed, giving more water and reproducing the mineral phosphate and some of the original sugar.

Yeast develops best in the presence of a plentiful supply of oxygen, although the process of alcoholic fermentation is not directly influenced by the oxygen supply. It appears that the reasons why ethyl alcohol is set free without being further oxidized in this case (even in the presence of plenty of oxygen) are (1) that yeast is but poorly supplied with oxidase and (2) that the alcohol diffuses out of the cells about as rapidly as it is formed. As the concentration of alcohol increases in the medium, the yeast cells ultimately become poisoned by the alcohol, and the fermentation process ceases altogether when the alcohol concentration reaches a magnitude of about 16 per cent.

Different kinds of yeast act somewhat differently. They may be separated and identified by several methods, as by the time required for the production of ascospores, by the forms of their giant colonies, etc. Many bacteria and moulds, as well as yeasts, produce alcoholic fermentation.

In the metabolism of yeasts and other alcoholic-fermentation organisms, the more complex carbohydrates are generally first hydrolyzed (as when cane sugar is acted on by the enzyme invertase) to form glucose sugar, and the latter is then fermented by zymase. Other substances, simpler than glucose, some of which may occur as intermediate products in the breaking down of the latter, may be fermented in a similar manner. Thus, pyrotartaric acid ($CH_3COCOOH$) forms carbon dioxide and acetic aldehyde (CH_3COH) under the influence of carboxylase, the aldehyde being subsequently reduced to ethyl alcohol (CH_3CH_2OH) by the action of reductase. The reductase enzymes act by transmitting hydrogen from one substance to another. The substance that supplies the hydrogen is a *reducing agent*, while the substance that receives the hydrogen is an *oxidizing agent*. The latter is called the *acceptor of hydrogen*. Reductase action may be illustrated by the fermentation of lactic acid, which may be pictured by means of the following equations, in which M represents a respiration pigment acting as hydrogen acceptor.

$$1.\ \underset{\text{Lactic Acid}}{CH_3\text{-}CHOH\text{-}COOH} + M = \underset{\text{Pyrotartaric Acid}}{CH_3\text{-}CO\text{-}COOH} + MH_2$$

$$2.\ \underset{\text{Pyrotartaric Acid}}{CH_3\text{-}CO\text{-}COOH} = CO_2 + \underset{\text{Acetic Aldehyde}}{CH_3\text{-}COH}$$

$$3.\ \underset{\text{Acetic Aldehyde}}{CH_3\text{-}COH} + MH_2 = \underset{\text{Ethyl Alcohol}}{CH_3\text{-}CH_2OH} + M$$

The hydrogen taken from the lactic acid is shown as finally added to the acetic aldehyde, the latter being reduced to ethyl alcohol. The hydrogen acceptor is not used up in the process. Reductase can not act unless both an oxidizer and a reducer are present.

3. Other Kinds of Fermentation.—*Lactic acid fermentation* (the ordinary souring of milk) is caused by the lactic acid bacillus, which grows in the presence of oxygen, and the same process is carried on by many other forms. It results in the hydrolytic splitting of lactose ($C_{12}H_{22}O_{11}$) into four times as many molecules of lactic acid ($C_3H_6O_3$), one molecule of water being consumed for each molecule of lactose decomposed. Other sugars (such as saccharose and maltose) are similarly decomposed with the formation of lactic acid, by other microörganisms.

Butyric acid fermentation occurs in the absence of oxygen and results in the formation of carbon dioxide and hydrogen, as well as of butyric acid. It is caused by certain forms of bacteria, especially species of Clostridium. Either glucose ($C_6H_{12}O_6$) or lactic acid ($C_3H_6O_3$) may be fermented in this way.

Very many other fermentation processes are known, due to numerous different forms of bacteria, etc., each organism being limited to certain kinds of fermentation.

4. Conditions Influencing Aerobic Respiration in Plants.—Ingen-Housz (1779) discovered that ordinary green plants respire like animals, taking in oxygen and giving off carbon dioxide, and DeSaussure made the first quantitative study of this process. The rate of gaseous exchange is greater for higher temperatures, up to about 40°C., above which the rate remains about the same until death occurs. Any change of temperature accelerates respiratory activity for a time. The value of the respiratory ratio (the amount of carbon dioxide given off divided by the amount of oxygen absorbed, in a given time period) is low (about .35 to .40) for temperatures about 10° or 15°C., and is progressively higher for either progressively higher or progressively lower temperatures. For temperatures about 35°C. this value was found to be .95.

Aerobic respiration in chlorophyll-bearing cells is apparently related to carbohydrate photosynthesis (which may be considered as the reverse of respiration), especially on account of the dependence of respiration on water-soluble carbohydrates. Of course the carbon dioxide produced by respiration in green leaves in sunlight is regularly fixed by the photosynthetic process. During periods of sunlight (other conditions being suitable) no carbon dioxide passes from green leaves to the surrounding air and no free oxygen enters the leaves; photosynthesis is then more active than respiration, and the net result of both processes is absorption of carbon dioxide and elimination of oxygen. The direct influence of light on the respiration rate may be readily studied in organisms and tissues without chlorophyll.

The partial pressure of oxygen in the surroundings influences the rate of respiration, since this pressure affects the rate of oxygen supply to the respiring cells. The supply of water and the osmotic value of the cell sap and of the environmental solutions, influence the respiration rate, as do also the rates of supply or partial concentrations of many other substances. Here may be mentioned phosphates, and many alkaloids, ethers, alcohols, aldehydes, etc. Respiration is accelerated by the presence of poisons (such as alcohols, ether) if these substances are supplied in the right concentration, which must be very weak. Wounding accelerates aerobic respiration.

The aerobic respiration rate in ordinary plants is closely related to the rate of enlargement; as the latter rate increases and then decreases, during the grand period of growth, the respiration rate alters in a similar manner. Actively enlarging tissues generally absorb somewhat more oxygen than they give off in the carbon dioxide eliminated; the value of their respiration ratio $\left(\frac{CO_2}{O_2}\right)$ is less than unity.

The nature of the material consumed in respiration largely determines the value of the respiration ratio. For germinating starchy seeds this value is about unity, but for germinating fatty seeds it is much lower. The following equations, for the complete oxidation of starch $[(C_6H_{10}O_5)_n]$ and for that of triolein (glycerine trioleate, the main fat in cotton seed, for instance), serve to show why oxygen accumulates in germinating fatty seeds and does not do so in germinating starchy seeds. Starch contains much more oxygen, in proportion to its carbon content, than does fat.

$$\underset{\text{Starch}}{C_6H_{10}O_5} + \underset{\text{Oxygen}}{6\,O_2} = \underset{\text{Carbon Dioxide}}{6\,CO_2} + \underset{\text{Water}}{5\,H_2O}. \quad \text{In this case } \frac{CO_2}{O_2} = \frac{6}{6} = 1.00$$

$$\underset{\text{Triolein}}{C_3H_5O_3(C_{18}H_{33}O)_3} + \underset{\text{Oxygen}}{80\,O_2} = \underset{\text{Carbon Dioxide}}{57\,CO_2} + \underset{\text{Water}}{52\,H_2O}. \quad \text{In this case } \frac{CO_2}{O_2} = \frac{57}{80} = 71$$

Ripening fruits in which fat is being formed from carbohydrates exhibit a respiration-ratio value much greater than unity.

5. The Measurement of Aerobic Respiration.—The rate of aerobic respiration may be measured in terms of the rate of oxygen absorption or in terms of the rate of carbon-dioxide elimination. The value of the respiration ratio is derived from the magnitudes of these two rates.

6. Respiration Water.—Water is produced by aerobic respiration, as has been said, but the rate of water formation is very difficult to measure and but few studies on respiration water have been reported. It will be remembered that hydrolytic processes (controlled by hydrolytic enzymes; see Chapter VII, Section 3) consume water and that the reverse processes liberate this substance. For example, saccharose combines with water and forms dextrose (glucose) and levulose; but if a molecule of dextrose and one of levulose unite to form a molecule of saccharose, a molecule of water is produced.

$$\underset{\text{Saccharose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} \rightleftarrows \underset{\text{Glucose}}{2\,C_6H_{12}O_6}$$

The formation of carbohydrates by photosynthesis consumes large amounts of water, one molecule of water for each atom of carbon fixed in the process. These, as well as other water-consuming or water-producing processes that occur in the plant, tend to hide the production of water by respiration.

7. Liberation of Heat During Aerobic Respiration.—The temperature of the plant body is generally very nearly the same as that of the surroundings, simply because the periphery conducts and radiates heat with so little resistance that no considerable temperature gradient between the environment and the tissues is generally maintained. The processes of respiration, including the final oxidations, liberate considerable amounts of energy within the active tissues, and much of this takes the form of heat, which tends to raise the tissue temperature. But this excess of heat is generally conducted to the surroundings about as rapidly as it is formed; consequently the internal temperature is usually only slightly, if at all, higher than the external. As has been noted, the absorption of solar radiation tends to raise the temperature of plant parts that are exposed to sunlight, during the periods of such exposure, and this is another source of increased heat within these parts. But this heat also is usually lost

about as rapidly as it is developed. Besides outward conduction (and some outward radiation) of heat, a very large part of the heat developed in the aerially exposed parts of plants disappears in the process of transpiration; it passes to the surrounding air without raising the temperature of the latter, for it becomes potential energy (the latent heat of water vapor). Rapidly transpiring leaves are generally a little cooler than the surrounding air, even in direct sunlight.

When there are large numbers of very active cells crowded into a small space, with not too ready heat conduction to the surroundings, the heat developed by respiration becomes evident, and the temperature of the tissue may be much higher than that of the surroundings. The internal temperature of an Arum spadix with opening flowers may be more than 25°C. higher than that of the surrounding air. A mass of germinating seeds or of opening leaf-buds may develop very high temperatures (7° to 20°C. higher than those of the surrounding air), especially if outward heat transfer is artificially hindered, as by enclosing the seeds in a Dewar flask. In germinating seeds the maximum rate of heat production occurs very early, just after germination begins, and this rate becomes lower as the seedlings develop. The highest rates of heat production appear to occur with the lowest values of the respiration ratio.

The heat produced by respiration is generally in excess of the amount calculated from the carbon dioxide given off or from the amount of oxygen absorbed, considering that carbon and oxygen simply unite to form carbon dioxide. A part of the difference is accounted for by considering the process as starting with carbohydrate and oxygen, instead of with carbon and oxygen. Not all the energy set free by respiration appears as heat; some of it disappears in the performance of the various kinds of work accomplished in the organism.

8. Anaerobic, or Intramolecular, Respiration.—In active plant tissues that usually require oxygen, anaerobic respiration continues for a time after the supply of oxygen has been cut off. As has been said, this part of the respiration process gives rise to incompletely oxidized carbon compounds, such as alcohols, acids, etc., as well as to some carbon dioxide or water, or both. If kept too long without oxygen supply, tissues finally die, being perhaps poisoned by the accumulation of incompletely oxidized products.

9. Respiration Chromogens and Pigments.—*Pro-chromogens* appear to be common in plant tissues. These are substances apparently of the nature of glucosides. They are decomposed by the glucoside enzyme emulsin, with the formation of corresponding *chromogens*. The latter seem to play an important rôle in aerobic respiration; they apparently unite readily with free oxygen under the influence of oxidizing enzymes, producing water and corresponding *respiration pigments*. These pigments then act as acceptors of hydrogen, uniting with the hydrogen produced by the anaerobic phase of respiration, and thus produce the corresponding chromogens once more. The pigments act as carriers of hydrogen, taking it up as it is produced (thereby becoming chromogens) and then delivering it to free oxygen, with the formation of water (and the re-formation of the pigments): pigment + hydrogen = chromogen; chromogen + oxygen = water + pigment.

10. Respiration Enzymes.—Plant tissues may be killed without destroying their enzymes, and they may then continue to give off carbon dioxide and to absorb oxygen, but in a somewhat different way from that exhibited by the living tissues. From studies on the respiration of such tissues, Palladin and others suggest that the carbon dioxide given off in aerobic respiration all arises from the anaerobic phase, while the water formed is a product of the union of free oxygen with respiration chromogens, in

the aerobic phase. It is supposed that sugar and water unite and form carbon dioxide and hydrogen, that the hydrogen unites with respiration pigments and thus forms chromogens, and that the chromogens are directly oxidized by free oxygen, forming water and the pigments. According to this hypothesis, as long as an unsatisfied acceptor of hydrogen is present, alcohol and similar substances are not formed, and the acceptor (pigment) is kept unsatisfied through the oxidation of the chromogen as long as free oxygen is adequately supplied. On the other hand, in the absence of free oxygen the pigment soon becomes satisfied and ceases to be able to absorb hydrogen, after which alcohol, etc., arise from the original decomposition of sugar, along with carbon dioxide.

11. **Materials Consumed in Respiration.**—In ordinary plants it is generally true that carbohydrates are the substances consumed by respiration. The respiration processes are controlled by enzymes, and these, in turn, are formed in the protoplasm. On the amount of protoplasm in a tissue depends the amount of enzymes present, and the latter determine the rate of respiration as long as the supply of carbohydrates is adequate. With inadequate supply of carbohydrates the respiration rate is low, even with plenty of protoplasm and enzymes; on the other hand, an excess of carbohydrates exerts no influence on the respiration rate. With plenty of carbohydrates it appears that the respiration rate varies with the amount of nucleins in the respiring tissue, the amount of nucleins being considered as proportional to the amount of complex proteins, insoluble in gastric juice. It may be supposed that the amount of complex proteins present is proportional to the amount of respiration enzymes, and this supposition may explain the apparent relation between the respiration rate and the supply of complex proteins. Neither simple nor complex proteins are generally used in respiration; carbohydrates may sometimes result from protein decomposition, however, and these may be used.

12. **Special Cases of Respiration in Lower Plants.**—In many lower forms, substances other than carbohydrates are decomposed in respiration. In acetic-acid bacteria, for example, respiration is the simple oxidation of ethyl alcohol (by means of free oxygen), with the formation of acetic acid and water. When the supply of alcohol has been consumed, however, these bacteria oxidize acetic acid and thus form carbon dioxide and water. Many other alcohols are similarly oxidized by microörganisms.

As already mentioned (Chapter II, Section 3), many bacteria obtain energy from inorganic substances, and it should be remarked that the decompositions thus brought about are respiration processes. Hydrogen sulphide, ammonia, hydrogen, etc., are thus oxidized.

13. **Circulation of Energy in Nature.**—While the amount of matter in and on the earth remains always practically the same, almost no material being now given off to, or received from, the rest of the universe, the energy exchange between the earth and its surroundings is very rapid and exceedingly important. Energy is continually and rapidly radiated from the earth's surface into sidereal space, and the supply available to organisms would soon be practically exhausted if it were not for the fact that new radiant energy is continuously being supplied from the sun. A very small part of the radiant energy emanating from the sun is intercepted by the earth, and a small portion of what is intercepted is rendered potential by the photosynthetic formation of carbohydrates in chlorophyll-bearing plant tissues. The potential energy of the carbohydrates thus formed becomes again kinetic through the processes of respiration, oxidation, and combustion. Most of this energy from carbohydrates is quickly radiated from the earth into the surrounding universe, and the remainder goes the

same way after it has taken part in the physical, chemical, and physiological processes that occur in organisms, and otherwise, on the earth's surface. The dissipation of solar energy is merely somewhat delayed by carbohydrate formation in chlorophyll-bearing tissues and by other processes of energy fixation that occur in plants.

PART II

PHYSIOLOGY OF GROWTH AND CONFIGURATION

CHAPTER I

GENERAL DISCUSSION OF GROWTH

§1. **Anatomical Relations of Cell Growth.**—Microscopical observation of the development of plant cells shows that three different stages of growth may be distinguished. The growth of the cell begins with its formation by division, this is the first stage of growth. The cell then begins to increase in size, thus passing into the period of enlargement, which is the second stage. Enlargement finally ceases, to be followed by thickening of the cell wall through the deposition of new layers of cellulose, and this constitutes the third stage of growth. The last two stages are not entirely distinct but merge gradually into each other, for deposition of new layers of cellulose occurs simultaneously with the enlargement of the cell. Fig. 96, a cross-section through the cambium region of the stem of the Scotch pine, shows all three stages in the development of tracheides from cambium cells. If all the cells of a tissue are in the first or in the third stage of growth, the growth changes characteristic of these stages are without effect upon the size of the tissue mass. In considering a tissue, these two stages may therefore be designated as stages of *internal growth*, as distinct from the second growth stage, that of enlargement, of which increase in the dimensions of the tissue or organ is the most characteristic feature.[a]

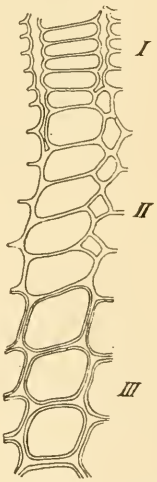


Fig. 96.—Cambium cells of Scotch pine, showing transformation into tracheides. The Roman numbers denote the three stages of growth.

[a] Not only is a sharp distinction between the second and third stages of growth impossible, as the author states, but the same is also true regarding the first and second stages; a certain amount of enlargement usually precedes each cell division in tissues that are accounted as in the first stage. The three stages furnish a convenient mode of reference, however, to the corresponding portions of the continuous march of the growth process. The first stage (called also the embryonic or formative phase) is mainly characterized by cell division, the second (called the phase of enlargement) is mainly characterized by cell enlargement, and the third (called the phase of maturation) is mainly characterized by thickening and other alterations in the cell walls, frequently also by changes of other sorts.—*Ed.*

Physiological studies of the rate of growth of a plant are generally carried out by measuring the part in question, either with a simple millimeter rule or with special measuring apparatus. In experiments of this kind only the external growth, or the enlargement of the plant, is measured, and the rate of enlargement is determined for a definite time period and under a certain set of conditions. Internal growth cannot be studied with a rule, it can be measured only by means of the microscope, or by qualitative and quantitative analyses of the materials found in the plant at different periods of its development.

§2. **Conditions Favorable to Growth.**—Growth of the cell is a result of the activity of protoplasm, and a large number of conditions must be fulfilled in order that it may take place. If a single one of the necessary external conditions be absent, then growth ceases, and if the internal conditions necessary for growth are not all fulfilled growth fails to occur in this case also, even though all other conditions are favorable.

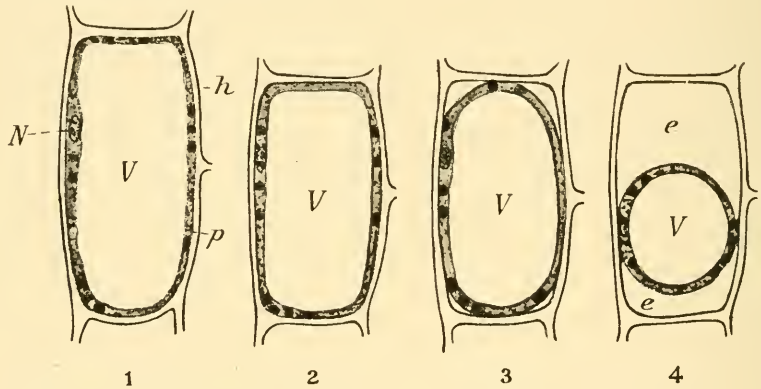


FIG. 97.—Different stages in plasmolysis of a cell. *N*, nucleus; *V*, vacuole. (*After de Vries.*)

Turgidity is one of the internal conditions necessary for cell enlargement. If a growing cell is placed in a 10 per cent. solution of sodium chloride, potassium nitrate or sugar, it immediately begins to decrease in size (Fig. 97). At first the cell wall and the protoplasmic membrane contract equally, but later, when the cell wall can contract no more, the protoplasm still continues to move inward, thus retreating from the cell wall. Finally, the entire contents of the cell collect into a ball-like mass in the center of the cell,with the outer protoplasmic membrane on the outside. This process is known as plasmolysis, as has been pointed out (page 114). If a plasmolyzed cell is placed in pure water it enlarges and finally regains its original size and form. The external conditions that produce these changes in cells are likewise effective in causing the shrinkage of an animal bladder filled with weak salt solution, when this is placed in a strong salt solution. The cell sap of plant cells is a solution of various substances, which have an attraction for water. The osmotic pressure produced in the cell when plenty of water is supplied results in the turgidity of the cell.

The enlargement of each cell begins with the stretching of the cell wall by

turgor, and the effect of this stretching becomes subsequently established by the deposition of new layers of cellulose. Traube's artificial cell is closely analogous to the living cell in some respects. If a drop of gelatine is introduced into a tannin solution, a precipitation membrane of gelatine tannate is formed at the surface of the drop, and the cell thus artificially produced begins to

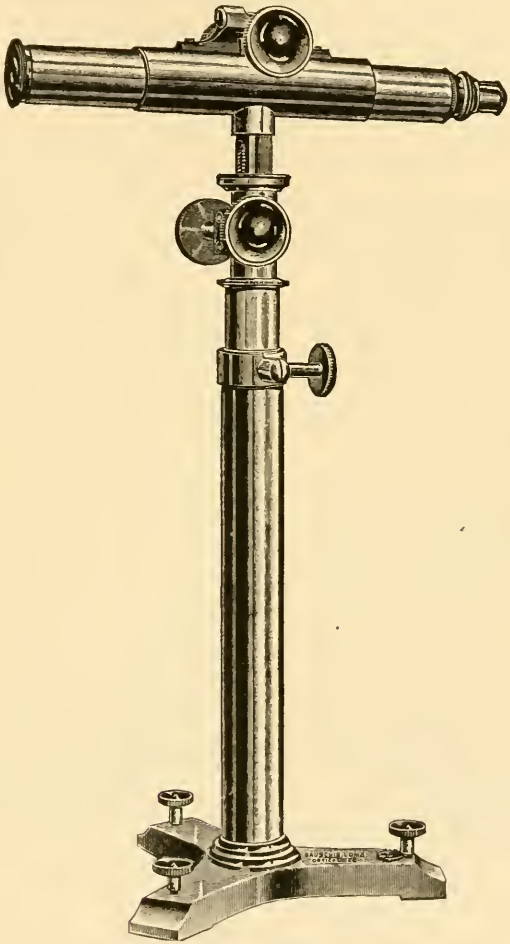


FIG. 98.—Horizontal microscope.

enlarge. This enlargement can be explained only by supposing that the gelatine extracts water from the tannin solution; the outward pressure thus produced causes a stretching of the membrane, which becomes ruptured at many places. Through the small openings thus formed the gelatine once more comes into contact with the tannin and the precipitation membrane is reformed, when the process is repeated.

Experiments with plasmolysis were at first conducted only with single cells, but de Vries[1] plasmolyzed entire plant organs during the period of enlargement. He showed that when pieces of the growing region, of stems, roots or flower-stalks, were placed in concentrated salt solution a considerable shortening was evident. This shortening is due to plasmolysis of the cells, and the plasmolyzed pieces were always wilted and flaccid, but when they were returned to pure water they regained their former length and rigidity. Mature organs, however, whose enlarging periods were over, showed no shrinkage when placed in strong salt solutions; the stretching caused by turgor had by this time become fixed through further deposition of cellulose on or in the walls.

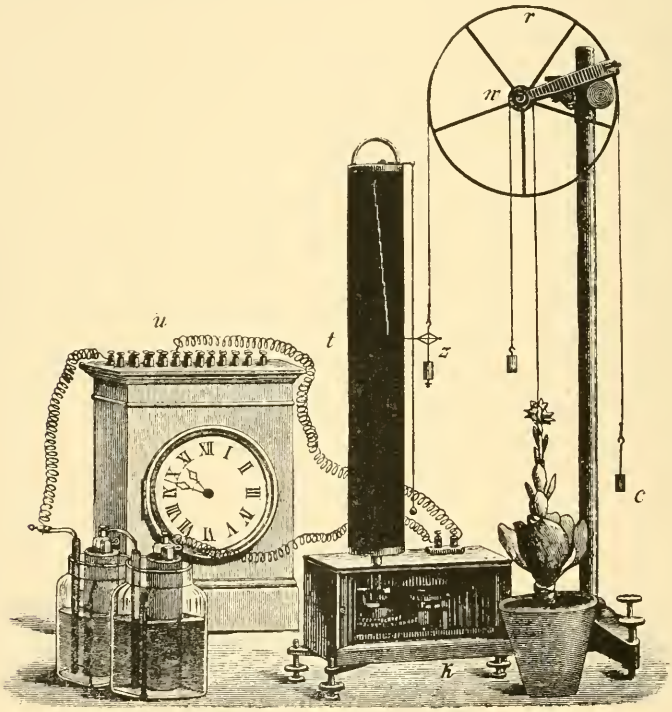


FIG. 99.—Auxanometer. (*After Pfeffer.*)

Turgor can thus produce enlargement of cells only when the walls are capable of being stretched by the pressure that is developed. Experiments carried out by Wortmann[2] showed that the cell walls of young cells possess this quality of extensibility in a much higher degree than do older ones, extensibility decreasing gradually with advancing age. The ultimate loss of this quality of the

[1] **Vries, Hugo de,** 1877, *1. 2.* [See note 1, p. 121.]

[2] **Wortmann, J.,** Beiträge zur Physiologie des Wachsthums. Bot. Zeitg. **47**: 229–239, 245–253, 261–272, 277–288, 293–304. 1889. **Schwendener, S.,** and **Krabbe, G.,** Ueber die Beziehung zwischen dem Maass der Turgordehnung und der Geschwindigkeit der Längenzunahme Wachsender Organe. Jahrb. wiss. Bot. **25**: 323–369. 1893.

walls results in the termination of cell enlargement, even though turgor may not have decreased. Extensibility of the wall is therefore the second condition necessary for cell enlargement. Various external conditions are also necessary for growth, such as favorable temperature conditions, the presence of oxygen in the surrounding air, and an adequate supply of water.

§3. **Apparatus for the Study of Growth.**—The simplest equipment for the study of plant enlargement is a millimeter rule. A horizontal microscope (Fig. 98) or a cathetometer may be used for finer and more accurate measurements. The auxanometer, a self-registering apparatus for growth measurement, may also be used (Fig. 99). A waxed thread is fastened to the top of the stem to be studied and is passed vertically upward and over a pulley, and a weight is attached to the free end. The pulley turns as the plant elongates and the weight descends. The growth increments are magnified by introducing a larger pulley, mounted on the same axis as the first, over which is passed a second thread with a weight at either end. A pointer is fastened to one end of the second thread, its tip resting lightly upon a vertically placed drum revolved by clockwork and covered with smoked paper. As the drum revolves and as the pulley turns with the elongation of the plant, a curve is traced on the paper, the slope of which represents the time rate of this elongation during the period of operation.

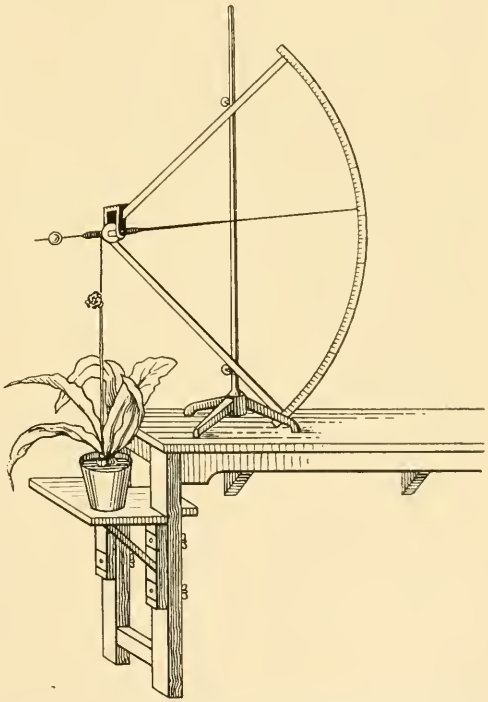


Fig. 100.—Apparatus for the study of growth.

If it is required to determine whether all parts of an organ grow with equal rapidity, the organ may be marked into millimeter or centimeter spaces or zones, by means of India ink. After some time the spaces are remeasured.

The apparatus shown in Fig. 100 may also be employed to study growth. One end of a waxed thread is attached to the tip of the plant and passes vertically upward, ending by being wound about a small pulley on a horizontal axis. To this pulley is attached a long, counterbalanced pointer, the free end of which moves upward or downward in front of a large graduated arc, as the pulley is turned. As the plant elongates the thread is released and the magnified growth increments are read directly in degrees of arc.

Summary

1. **Anatomical Relations of Cell Growth.**—The first stage, or phase, of cell growth is that of cell *division*—this being also called the *formative*, *meristematic*, or *embryonic* phase. After its formation by division each cell enters the second phase, that of *enlargement*, in which it attains its mature size. After enlargement ceases various changes occur leading to the condition of maturity, such as the thickening of walls, etc., and these changes characterize the third phase, that of *maturation*. The three growth phases cannot be sharply distinguished, however.

Measurements of growth rates frequently refer only to the second phase, that of enlargement, since this is the most easily studied by means of a millimeter rule, etc. Microscopic methods are resorted to in studies of all three phases, growth in general is frequently measured in terms of increase in weight, and chemical determinations of the various substances formed are often employed, especially for studies of the third phase.

2. **Conditions Favorable to Growth.**—Growth is a complex physiological process, being the resultant of many component processes, and—like all other processes—it cannot occur unless all the essential conditions are fulfilled. Some of the essential conditions are internal (within the plant body), while others are external (in the environment). One of the primary internal conditions necessary for cell enlargement is *turgidity*, which is produced by osmotic pressure or imbibition pressure within the cell. By this pressure the protoplasm is held against the cell wall and the latter is more or less stretched. Cell enlargement is primarily due to osmotic and imbibition pressures developed through the absorption of water, and to the resultant stretching of the cell wall. New cellulose is deposited in the stretched wall and more stretching occurs, with still further addition of cellulose, until the second phase of growth is completed. In the third phase of growth the cell wall often thickens on account of deposition of cellulose without further stretching.

The importance of turgidity is shown experimentally by plasmolysis and the recovery therefrom. Artificial osmotic cells, especially Traube's artificial cell (a drop of gelatine solution in a solution of tannin), illustrate some of the phenomena of turgor and plasmolysis. When enlarging plant organs or tissues are placed in properly concentrated salt or sugar solutions a pronounced contraction results, due to the removal of the turgor pressure within the cells. The wilted tissue returns to its original turgid and stretched condition after the concentrated solution has been replaced by water or by a very weak solution. After the second phase of growth has been completed plasmolysis of the cells results in little or no contraction of the tissue; in the third phase of growth the cell walls are stretched but little or not at all. Extensibility of the cell wall, under the influence of the pressure of turgor that occurs in the cell, is therefore another internal condition necessary for cell enlargement.

Many external conditions are also necessary for growth, such as temperatures within certain limits, proper oxygen supply, water supply, etc.

3. **Apparatus for Studying Plant Enlargement.**—The enlargement of an organ or plant may be measured by various devices, from the simple metric scale to very precise auxanometers, auxographs, etc. Equally spaced marks may be made upon the surface of an enlarging organ and subsequent determination of the distances between the marks indicates the relative rates of enlargement of the various regions of the organ.

CHAPTER II

GROWTH PHENOMENA THAT ARE CONTROLLED BY INTERNAL CONDITIONS

§1. The Grand Period of Growth.—Plants and their component organs and tissues do not enlarge at the same rate throughout the period of their development. Enlargement begins at a slow rate, which gradually increases until a maximum is reached, after which the rate progressively decreases until enlargement ceases altogether. The time period corresponding to this march of rate of enlargement was designated by Sachs[1] as the grand period of growth, and the same author called the graph representing this march, the grand curve of growth. This peculiar march of the growth rate is due to the fact that each individual cell of the plant body passes through a similar grand period of development. External conditions can lengthen or shorten the period of growth, but the general character of the curve is not altered. Thus, in one experiment, the daily increments of elongation of the terminal internode (3.5 mm. in length) of a seedling of *Phaseolus multiflorus*, with a temperature of 12.8 to 13.8°C., had the values shown below.

Number of Day	Increment of Elongation *mm.*
1	1.2
2	1.5
3	2.5
4	5.5
5	7.0
6	9.0
7	14.0
8	10.0
9	7.0
10	2.0

§2. Growth of Root, Stem and Leaf.—While it is generally true that the three most important organs of the plant (roots, stems and leaves) all pass through a grand period of growth, nevertheless there are individual peculiarities to be observed in each case.

In roots,[2] the elongating region is restricted to a portion near the tip, usually not more than 10 mm. when the roots are surrounded by soil. Aerial roots are an exception to this; the elongating regions of the aerial roots of *Monstera*

[1] **Sachs. J.,** Ueber den Einfluss der Lufttemperatur und des Tageslichts auf die stündlichen und täglichen Aenderungen des Längenwachsthums (Streckung) der Internodien. Arbeit. Bot. Inst. Würzburg 1: 99–192. 1874.

[2] **Sachs, J.,** Ueber das Wachsthum der Haupt- und Nebenwurzeln. Arbeit. Bot. Inst. Würzburg 1: 385–474, 584–634. 1874.

deliciosa are about 30 to 70 mm. in length, while those of *Vitis velutina* may exceed 100 mm. The individual parts of the region of elongation in the root show unequal rates of growth. The most rapidly elongating portion lies in the center of the region, while the parts above and below grow more slowly. An experiment in which young roots of *Vicia faba* seedlings were divided (by India ink lines) into ten zones each a millimeter long, the zones being measured after twenty-four hours, gave the following values for the increments of elongation of the respective zones. The temperature was 20.5°C. and the zones were numbered from the tip upward.

Number of Zone	Increment of Elongation *mm.*
X	0.1
IX	0.2
VIII	0.3
VII	0.5
VI	1.3
V	1.6
IV	3.5
III	8.2
II	5.8
I	1.5

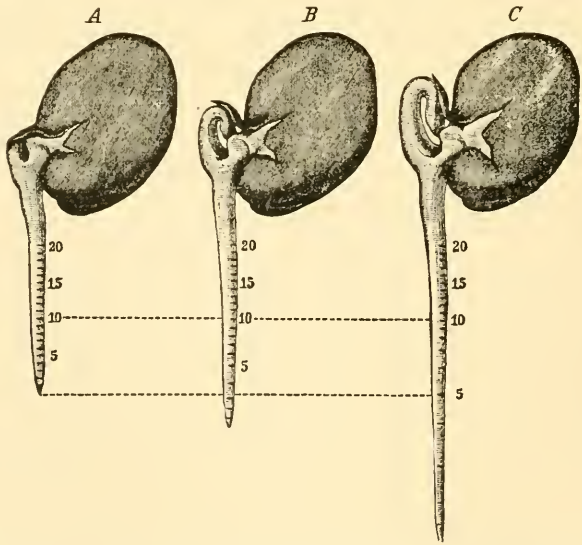


Fig. 101.—Three stages in the elongation of a root of *Vicia faba*.

In Fig. 101 are shown three stages in the elongation of the primary root of a *Vicia faba* seedling. *A* shows the root divided into millimeter zones, at the beginning of the experiment, and *B* and *C* show the same seedling after six hours and after one day, respectively.

Each zone of the elongating region of the root likewise passes through a grand period of growth. In an experiment in which the primary root of a seedling of *Vicia faba* was marked into millimeter zones, each zone being measured after one, two, three, etc., days (the temperature being from 18° to 21.5°C.), the following daily increments of elongation of the youngest zone were observed.

Number of Day	Increment of Elongation *mm.*
1	1.8
2	3.7
3	17.5
4	17.5
5	17.0
6	14.5
7	7.0
8	0.0

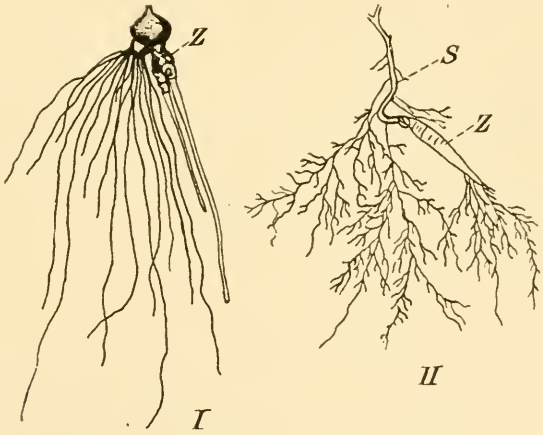


Fig. 102.—I, *Crocus longiflorus*. II, *Oxalis*. Z, contracting roots. Half natural size.

Neither does the stem[1] enlarge throughout its entire length at the same time, but the elongating regions are here much longer than in the root. The stem of *Galium molligo* has a terminal region of elongation from 2 to 4 cm. long, embracing from 8 to 10 internodes; this region in *Aristolochia sipho* is from 40 to 50 cm. long and embraces from 8 to 10 internodes; in *Elodea canadensis* it is from 2 to 3 cm. long, with from 43 to 50 internodes; and in *Hippuris vulgaris* it is from 20 to 30 cm. long. The single individual zones of the stem, as is true also of the root, elongate unequally, and each passes through a grand period of growth.

Leaf enlargement[2] is mainly basipetal, the enlarging region being situated in the lower portion of the organ, near the stem. In the table below are given the

[1] **Askenasy, E.**, Ueber eine neue Methode, um die Vertheilung der Wachsthumsintensität in wachsenden Theilen zu bestimmen. Verhandl. Naturhist.-Med. Ver. Heidelberg 2: 70–153. 1880.

[2] **Stebler, F. G.**, Untersuchungen über das Blattwachsthum. Jahrb. wiss. Bot. 11: 47–123. 1878.

daily increments of elongation in a leaf of *Allium cepa* (onion), at different stages of its development, with a temperature of from 19 to 21°C. The leaf was divided into 2.5-mm. zones, and these zones are here numbered I to IX, beginning with the basal one. The experiment began on March 8, and the increment of each zone was determined after one day. The average daily increments were again determined for the period from March 16 to 18, and finally for the period from March 22 to 23.

	Number of 2.5-mm. Zone	Average Daily Increment of Elongation			Total Increment of Elongation, March 8–23
		March 8–9	March 16–18	March 22–23	
		mm.	*mm.*	*mm.*	*mm.*
Leaf sheath	I	0.1	0.0	0.0	7.9
	II	0.1	2.9	0.0	26.4
Leaf blade	III	0.1	2.9	0.2	25.1
	IV	0.4	5.1	0.1	48.1
	V	0.4	3.0	0.0	30.1
	VI	0.2	2.1	0.0	19.1
	VII	0.2	1.6	0.0	16.7
	VIII	0.2	0.7	0.0	10.4
	IX	0.1	0.8	0.0	1.4
Total for entire leaf	.....	1.8	18.3	0.3	185.1

It is evident from these data that elongation soon ceased in the upper part of the leaf (zone IX), and that the greatest elongation occurred in the lower and younger part.

Growth may sometimes result in a shortening, instead of an elongation.[1] This may arise from active growth of the parenchymatous cells of the cortex, in a radial direction, in which case the vascular bundles assume an undulating form. Shortening is sometimes pronounced, and it frequently has great biological significance where it occurs. Many roots shorten or contract longitudinally and thus draw the buds, located above, down into the soil, so that the latter are protected from wounding and shielded from injurious atmospheric conditions. In the case of *Arum maculatum*, the little tubers formed at a depth of 2 cm. are subsequently drawn into the soil to a depth of 10 cm. If the tubers are planted less deeply, strongly contractile roots are soon formed, which draw them deeper into the soil. In the case of *Crocus longiflorus* (Fig. 102), only slender roots are formed in the spring. Thick lateral roots with great contracting power are formed later, and these drag the corm downward to a considerable depth, and then wither away.

[1] Vries, Hugo de, Ueber die Kontraktion der Wurzeln. Landw. Jahrb. 9: 37–80. 1880.

§3. **Tissue Strains.**[a]—Each plant organ consists of many kinds of tissues, and the different sorts of cells do not divide and enlarge at a uniform rate. It thus follows that opposing forces, or stresses, develop between the tissues, one tissue pressing against another while the latter, in its turn, also tends to enlarge and press against the former. Thus result what are called tissue strains, which increase the rigidity of plant organs. In every plant some organs are in a state of strain by traction (*i. e.*, they are stretched), while others are under pressure (*i. e.*, they are compressed). Strains may occur either longitudinally or transversely. Longitudinal strains may be easily demonstrated. Two longitudinal cuts are made, perpendicular to each other, through the center of a dicotyledonous stem or the flower stalk of one of the Liliaceæ or of Taraxacum (dandelion), which is still elongating. The four strips of stem thus formed bend outward, the originally outer surface becoming concave. From this it follows that the epidermis and cortex are stretched in the uncut stem, while the pith is compressed. Splitting the stem allows the pith to expand and the cortex to contract. Each concentric layer of tissue in an internode that is elongating is stretched with respect to the next layer within and compressed with respect to the next external layer. If the strips just mentioned are placed in water the bending becomes more pronounced, and frequently results in coiling.

Transverse strains may be seen best in old stems of dicotyledonous plants. These strains are produced by the occurrence of more rapid enlargement in the wood than in the bark, so that the latter is stretched and the former compressed. If a girdling band of bark is removed from such a stem (willow, for example), and if it is then returned to its original position, the two ends fail to meet, because of the fact that the band contracted as it was removed.

Summary

1. **The Grand Period of Growth.**—The enlargement of a plant, organ, or tissue begins at a slow rate, and the rate gradually increases to a maximum, after which it progressively decreases until enlargement ceases altogether. The time period corresponding to this march of the elongation rate is called the grand period of enlargement. Each cell has its grand period. The influence of external conditions may lengthen or shorten the grand period or may alter the maximum rate of enlargement, but the general march of the rate is essentially controlled by internal conditions. For example, the terminal internode of a seedling bean elongated 1.2 mm. on the first day, 14 mm. on the seventh day, and 2 mm. on the tenth day.

[a] The word *strain* is here used in its mechanical sense, meaning any sort of *deformation*, whether of *tension* (enlargement), of *compression* or of *shearing* (changes of shapes without any change of volume). Many writers of English still use *tension* where *strain* is here employed, being thus led to the awkward teutonicism by which compression is called *negative tension*. It may be remarked that the *force* that tends to produce any kind of strain (whether actual deformation occurs or not) is to be called a *stress*, so that there are three kinds of stress corresponding to the three kinds of strain above mentioned. In this connection, see Ewart's remarks in v. 2, p. 62, footnote 1, of: **Pfeffer, W.**, The physiology of plants. Translated by A. J. Ewart, Oxford, 1903.—*Ed.*

2. **Enlargement of Roots, Stems, and Leaves.**—Elongation in roots is confined to a region near the tip. Different parts of the elongating region exhibit different rates of enlargement; the most rapidly elongating portion is near the middle of the region, while the parts above and below enlarge more slowly. The tip millimeter of a root of Windsor bean elongated but 1.5 mm. in 24 hours, while the third millimeter from the tip elongated 8.2 mm., and the tenth elongated only 0.1 mm. The tip millimeter of another root of the same kind elongated 1.8 mm. during the first day, 17.5 mm. during the fourth day, 7 mm. during the seventh day, and not at all during the eighth day.

In stems, elongation is generally confined to a few internodes near the tip, and each elongating internode exhibits different rates of enlargement for its different regions. In onion leaves growth is confined to the basal portion in all but very young specimens.

Growth may sometimes result in the *contraction* of an organ, as in the contractile roots by which Crocus corms are pulled downward in the soil.

3. **Tissue Strains.**—Since all parts of a plant organ do not enlarge at exactly corresponding rates, and since the various parts are all rather firmly joined, some tissues become stretched and others compressed, by adjacent tissues. Strains may thus be developed, in any direction, and they result in increased rigidity of the organ. Each concentric layer of tissue in an elongating internode is stretched with respect to the next layer within and compressed with respect to the next layer outside. The bark of an enlarging willow shoot shows transverse stretching, while the inner part of the shoot is compressed.

CHAPTER III

INFLUENCE OF EXTERNAL CONDITIONS ON GROWTH AND CONFIGURATION[1]

§1. Dependence of Growth and Configuration upon Temperature.—Medium temperatures[2] are most favorable for growth, which ceases with very high and with very low temperatures. The following table shows the increments of elongation of three plants for a forty-eight hour period, at various temperatures.

Temperature	Lupinus Albus (Lupine)	Pisum Sativum (Pea)	Triticum Vulgare (Wheat)
deg. C.	*mm.*	*mm.*	*mm.*
14.4	9.1	5.0	4.5
17.0	11.0	5.3	6.9
21.4	25.0	25.5	41.8
24.5	31.0	30.0	59.1
25.1	40.0	27.8	59.2
26.6	54.1	53.9	86.0
28.5	50.1	40.4	73.4
30.2	43.8	38.5	104.9
31.1	43.3	38.9	91.1
33.6	12.9	8.0	40.3
36.5	12.6	8.7	5.4

The minimum, optimum and maximum temperatures for the growth of several different plants are shown in the next table. This table shows clearly

	Minimum	Optimum	Maximum
	deg.C.	*deg. C.*	*deg.C.*
Hordeum vulgare (barley)	5.0	28.7	37.7
Sinapis alba (white mustard)	0.0	21.0	28.0
Lepidium sativum (garden cress)	1.8	21.0	28.0
Phaseolus multiflorus (scarlet-runner bean)	9.5	33.7	46.2
Zea mays (maize)	9.5	33.7	46.2
Cucurbita pepo (gourd, squash)	13.7	33.7	46.2

[1] [On plant movements in general, see: **Pringsheim, Ernst G.,** Die Reizbewegungen der Pflanzen. 326 p. Berlin, 1912.]

[2] **Köppen, W.,** Wärme und Pflanzenwachsthum. Bull. Soc. Imp. Nat. Moscou 43^{II}: 41–110. 1870. **Sachs, J.,** Physiologische Untersuchungen über die Abhängigkeit der Keimung von der Temperatur. Jahrb. wiss. Bot. 2: 338–377. 1860. [**Lehenbauer, P. A.,** Growth of maize seedlings in relation to temperature. Physiol. res. 1: 247–288, 1914. Also see: **Fawcett, H. S.,** The temperature relations of growth in certain parasitic fungi. Univ. Calif. Pub. Agric. Sci. 4: 183–232, 1921.]

that the minima, optima and maxima of temperature are not the same for different plants. The differences between the various minima are especially striking. Whereas growth of some plants is terminated at from 10° to 15°C., other plants are still able to develop at 0°; thus Soldanella (an alpine plant of the primrose family) begins to develop in the spring when the plants must break through the snow before the shoots reach the air.

Still more striking variations in minimum and maximum temperatures may be observed in microörganisms. Bacteria are known, for instance, that not only live, but multiply vigorously, at 0°C. In sea water at 0° have been found as many as 150 bacteria per cubic centimeter. If such water is allowed to stand without change of temperature, this number increases to 1750 in four days, which shows that bacteria continue to reproduce at the temperature of the freezing point of water.

Bacillus thermophilus is very different from the bacteria just mentioned, being able to reproduce actively at 70°C. While the optimum temperature for most bacteria lies between 10 and 15°, *Bacillus thermophilus* ceases to reproduce at temperatures below 42°.

Bacterial spores can endure great extremes of temperature, some being able to withstand a short period of exposure in liquid oxygen at-213°C. The spores of some soil bacteria can bear very high temperatures, but the higher the temperature is, the shorter is the time required to kill the spores. The time periods required to kill such spores in steam at various high temperatures are given below.

Temperature of Steam, *deg.C.*	Time Required to Kill, *hours*
100	16
105–110	2–4
115	0.5 –1.0
125–130	0.08
135	0.02–0.08
140	0.02

Temperature affects the configuration as well as the rate of enlargement of plants. In polar regions and on high mountain-tops, where the temperatures are low, it is usual to find plants very short and lying very close to the soil. It has been observed that the soil of high mountains is relatively much warmer than the air, and plants that remain close to the soil are thus in a warmer environment than would be the case if their stems extended up into the air. Moreover, these low forms are covered in winter with a deep layer of snow, which protects them from freezing. The stems and branches of *Pinus humilis* do not grow vertically into the air but occupy a horizontal position. Even trunks as much as 20 cm. in diameter, which might quite well support a broad top if they had a vertical position, lie almost horizontal upon the soil surface. So much for the observed facts, but experiments are needed for more definite knowledge. Recently, it has been possible to show that changes in tempera-

ture alone, other conditions being equal, are sufficient to produce differences in the external appearance of certain plants. Thus, for example, the stem of *Mimulus tilingii* grows vertically upward at ordinary temperatures, while it bends or even assumes a horizontal position at lower temperatures.

It is well known that the climate of high mountains is characterized by great *fluctuations* in temperature, and the question arises whether this environmental feature does not also play a part in producing the peculiar aspect of alpine plants. To answer this question by experiment, plants from low altitudes were grown from the seed in vessels that were surrounded with ice at night and were exposed to the usual lowland conditions during the day, thus simulating the daily temperature fluctuation observed on high mountains. Plants thus grown possessed the special peculiarities of the forms occurring in alpine floras (limited enlargement, short internodes, small, tough leaves, and early flowering periods).

A striking example of the influence of temperature upon plant configuration is found in the case of a species of acetic acid bacterium (*Bacterium pasteurianum*).

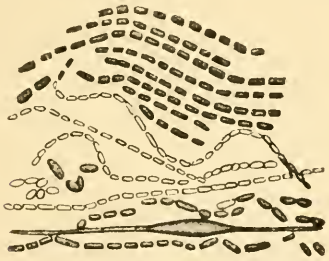


FIG. 103.—*Bacterium pasteurianum*, grown at 34°C.

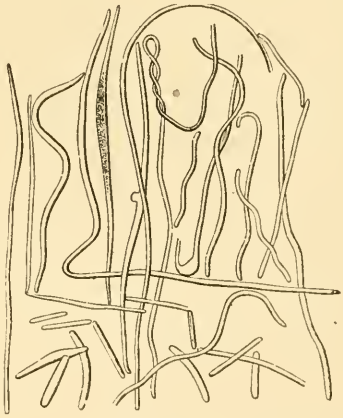


FIG. 104.—*Bacterium pasteurianum*, grown at 40°C.

Cultivated at medium temperatures, this organism assumes the form of short rods, usually joined together in rows or chains (Fig. 103). If a part of such a culture is transferred to fresh nutrient solution and subjected to a temperature of 40°C., the cells elongate, after a few hours, into slender filaments (Fig. 104). These filaments are sometimes as much as 150 times as long as are the original rod-shaped forms. When such a filamentous culture is returned to a temperature of 34°, the rod form is once more produced; the filaments first develop local swellings and then the portions between these thickened regions divide into the short cells of the other form. The thickened portions remain unchanged, and finally die.

The dependence of development upon temperature can be established by phenological observations. To find out the temperature requirements of any annual plant, the average or maximum temperature, above zero, is recorded for every day from the time of planting until the complete ripening of the fruit. The sum of these daily temperatures is taken to represent the amount of heat necessary for the complete development of the plants in question.

It is self-evident that such methods of observation can give but inaccurate and merely approximate results.[a] Plant growth is not proportional to temperature. On a certain day, for example, a temperature of 35° may occur, while the best temperature for the growth of the plant in question may be 25°. The additional 10° may not only be useless in promoting growth but it may even be injurious to the plant. Because a plant has developed under conditions giving a certain sum of daily temperatures, it is not safe to conclude that the same plant might not have developed equally well under conditions giving a smaller temperature summation. The birch grows near Kiev at a higher temperature than it experiences in the neighborhood of Petrograd. The following table, by which the course of development of the vegetation at Brussels and at Petrograd are compared, substantiates this conclusion. Six groups of plants are considered, the first group consisting of the earliest-flowering plants (Anemone, Corylus) and the other groups being composed of progressively later-flowering forms. The temperature measurements were begun in Brussels on Jan. 16, and in Petrograd on Apr. 8. The date of flowering for Brussels is given for each group of plants and also the number of days between this date and the corresponding date for Petrograd. The temperature summations, above 0°C., are also given for each group at the two stations, up to the time of flowering in each case.

Group No.	Date of Flowering at Brussels	Difference between Dates of Flowering at Brussels and at Petrograd	Summation of Daily Temperatures Above 0°C.	
			At Brussels	At Petrograd
		days	*deg. C.*	*deg. C.*
1	Mar. 16	51	184	93
2	Apr. 7	44	334	216
3	Apr. 29	39	583	421
4	May 19	33	791	617
5	June 4	22	1017	698
6	June 30	11	1466	937

These observations show that the plants at Petrograd came to flowering with a smaller temperature summation than did those at Brussels. It is also noteworthy that the date of flowering at the northern station is very markedly later than that at the southern only in case of the early-flowering forms, and that the

[a] On the general problem of integrating temperature values to obtain a measure of the effectiveness of temperature conditions for plant growth and development, see: **Livingston, B. E.**, Physiological temperature indices for the study of plant growth in relation to climatic conditions. Physiol. res. **1**: 399–420. 1916. Other references are there given. Also see: **McLean, F. T.**, A preliminary study of climatic conditions in Maryland, as related to plant growth. *Ibid.* **2**: 129–208. 1917. **Hildebrandt, F. M.**, A physiological study of the climatic conditions of Maryland as measured by plant growth. *Ibid.* **2**: 341–405. 1921. It must be remembered that many environmental conditions besides temperature are influential in determining plant behavior, and that these also vary from day to day and from place to place. Blackman's discussion of limiting conditions for plant processes has a bearing on this general problem. See: **Blackman**, 1905, 1908. (See note *w*, p. 35.) See also: **Livingston, B. E.**, and **Shreve, Forrest**, The distribution of vegetation in the United States, as related to climatic conditions. 16 + 590 p. Carnegie Inst., Washington, Publ. 284. 1921.—*Ed.*

difference between the two dates decreases as the date for Brussels becomes later; the difference is only eleven days in the case of the latest-flowering forms here considered, the linden being one of this group. This last observation may be explained by pointing out that the period with temperatures below the freezing point of water is also important in the development of perennials. This is a period of low activity, but not one of complete inactivity, and various chemical transformations are completed during the cold winter, which prepare the plant for the active growth of spring. These transformations are accelerated only very slightly by higher temperatures, as may be seen in the case of the sixth group considered above. The linden began to flower at Brussels only eleven days earlier than at Petrograd, although the temperature at the southern station was already above zero by the middle of January, and zero was not passed as Petrograd until early April. Direct experiment shows that higher temperature alone is not sufficient to bring plants out of the resting condition into active growth. In an experiment in this connection twigs were removed from a cherry tree at intervals throughout the winter and placed in a greenhouse with a temperature of from 20 to 25°C. Twigs cut in the autumn failed to produce leaves or flowers and finally died, while those cut during the winter and early spring flowered after they had been exposed to the greenhouse temperature for a certain time, this period becoming shorter with the advance of the season. The number of days of greenhouse conditions required to produce flowers on these twigs is shown below, for twigs cut at various dates. In spite of the favorable temperature of the greenhouse, the earlier the twigs were cut, the longer was the period before flowering.

Date of Cutting and Placing in Greenhouse	Period Required to Produce Flowers
	days
Dec. 14	27
Jan. 10	18
Feb. 2	17
Mar. 2	12
Mar. 23	8
Apr. 3	5

This experiment shows that, in making an estimate of the amount of heat necessary for development of the plant, it is necessary to consider the resting period which may continue, or even begin, in spite of temperature conditions generally favorable to active growth. Certain trees and shrubs, when transferred from temperate to warm climates and thus removed from the conditions of their winter resting period, although adequately supplied with moisture and heat (so that vital activity need not be directly retarded), still retain their earlier habit for a long time, losing their leaves and passing over into the resting condition for a part of the year. The life of the plant is thus not governed entirely by the amount of heat received; the internal conditions of the plant must also be considered.[b]

[b] In this connection, see: **Klebs, G.,** Ueber das Treiben der einheimischen Bäume speziell der Buche. Abhandl. (math.-naturw., Kl.,) Heidelberg. Akad. Wiss. 3: 1–116. 1914. This author has succeeded in overcoming the tendency to become dormant, by the control of culture conditions.—*Ed.*

As Molisch[1] has shown, even though the resting period may not be terminated by subjecting the plant to medium temperatures, it can be brought to a close by application of high temperature, especially if the branches to be forced are immersed for from ten to twelve hours in water at 30° to 35°C. or above. Fig. 105 shows a hazel branch the right side of which was subjected to Molisch's warm-bath treatment, while the left side was untreated. Nine days after the treatment the right side was already in full bloom, while the buds on the left side were still in the resting condition.

§2. Dependence of Growth and Configuration upon the Oxygen Content of the Surroundings.—Higher plants usually grow only when they may absorb oxygen; when the oxygen supply is cut off growth is immediately stopped. Nobakikh[2] has shown, however, that when certain conditions are fulfilled seed-plants may be made to grow in an atmosphere free from oxygen. He placed the plants in a solution of glucose. A double object was thus attained: the plants were furnished with nutrient material and, at the same time, the products of fermentation harmful to growth were allowed to pass into the solution. These results were later substantiated by other authors. As has been pointed out above (page 214), the amount of oxygen absorbed by germinating seedlings increases as the growth rate becomes more rapid. The march of the respiration rate in germinating seeds may be expressed by a grand curve of respiration which agrees, in general, with the grand curve of growth (see page 241).

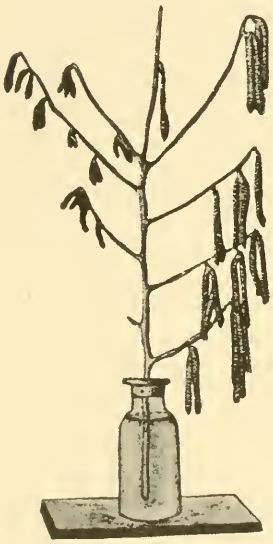


Fig. 105.—Effect of dipping resting buds in warm water; the right side of the branch was so treated.

The amount of oxygen contained in the surrounding atmosphere exerts a marked influence upon the rate of growth. An excess of this gas, as well as a deficiency, decreases the growth rate and may even cause growth to cease entirely. On the other hand, if the pressure of the air does not vary too far from the normal, in either direction, then such a change produces an acceleration of growth. This brings out the very noteworthy fact that growth under normal atmospheric pressure is less rapid than when the pressure is somewhat higher or somewhat lower than normal.[3]

It appears that oxygen is one of the most important factors in the life of microörganisms. For some organisms oxygen is essential, others can exist a long time without it, and still others can reproduce only under conditions where it is entirely absent (see Part I, Chapter VIII). Microörganisms may thus be separated into aerobes and anaerobes, according to their oxygen requirement.

[1] **Molisch, H.**, Das Warmbad als Mittel zum Treiben der Pflanzen. Jena, 1909.

[2] **Nabokikh, A. I.**, Temporary anaerobiosis of higher plants. [Russian.] Dissert. New Russia Univ., St. Petersburg, 1904. **Nabokich, A. J.**, [*Idem*], Temporäre Anaërobiose höhere Pflanzen. Landw. Jahrb. 38: 51–194. 1909.

[3] **Jaccard, Paul**, Influence de la pression des gaz sur le développement des végétaux. Rev. gén. bot. 5: 289–302, 348–354, 382–388. 1893.

Aerobes require oxygen for their development, while anaerobes can develop in the complete absence of this gas. Anaerobes are either obligate or facultative. Obligate anaerobes reproduce only when oxygen is entirely absent; it acts upon them as a poison. Facultative anaerobes are not seriously injured by oxygen and they also thrive in its absence. Acetic acid bacteria may be mentioned as an illustration of aerobes; yeasts, of facultative anaerobes; and the bacteria of butyric acid fermentation are obligate anaerobes.

Motile bacteria may be used as an indicator of the relative amounts of oxygen present in different regions of a mass of nutrient medium. In Fig. 106, respiration figures for three different kinds of bacteria are shown. In each case a drop of the culture was placed upon a slide and covered, the circular cover glass being raised at one edge by a bit of platinum wire. The drop of liquid thus came to lie under the half of the cover that was nearest to the slide. The first figure (I) shows the behavior of typhus bacteria, which are aerobes. The moving cells are most numerous in the region of the drop that contains the most oxygen. Those in the zone *r* have ceased moving because of deficiency of oxygen, while

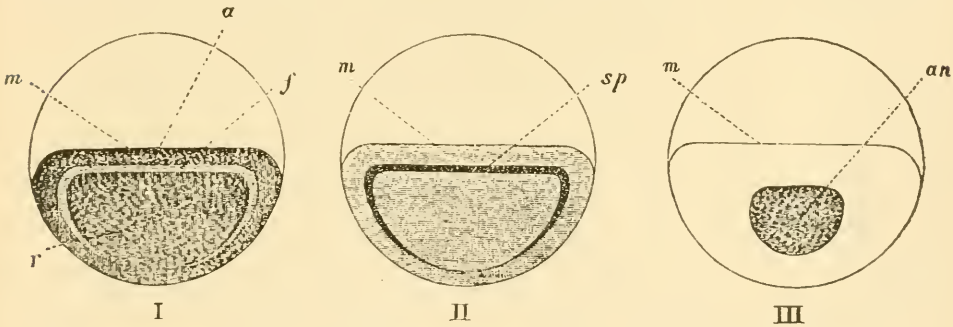


FIG. 106.—Respiration figures of motile bacteria. (*After Beijerinck.*)

the zone *f* is free from bacteria. The next figure (II) represents the distribution of spirillum, bacteria, which require a small amount of oxygen. The cells have collected in the zone *sp*, a certain distance from the free surface of the liquid. The third figure represents the activity of anaerobes in this sort of mounting. All the cells have collected in the central zone of the drop, *an*, where oxygen is least plentiful.

In the culture of anaerobes it is essential that precautions be taken to prevent the access of oxygen to the nutrient medium. For this purpose Pasteur employed a layer of oil over the nutrient solution.[c] The air may also be pumped out of the vessels in which the cultures are grown, or the oxygen may be absorbed from the air of the culture vessels by means of a solution of pyrogallol and potassium hydroxide. The test-tube containing the culture is placed within another larger test-tube, which is partially filled with alkaline pyrogallol (*P*, Fig. 107). The larger tube is tightly closed with a rubber stopper, the

[c] The effect of an oil layer in lowering the rate of oxygen supply to the liquid below depends upon the kind of oil used as well as upon the thickness of the layer. It must not be assumed that such an oil layer cuts off the supply of oxygen entirely. Of course the contents may be placed in a chamber of nitrogen or hydrogen, etc.—*Ed.*

oxygen is absorbed by the alkaline pyrogallol, and the bacteria of the inner tube are thus exposed to an atmosphere without oxygen.

The form of the plant may also be controlled by the oxygen content of the surroundings. Thus Mucor, a very common mould, develops a much-branched mycelium in the presence of oxygen, and produces vertical sporangiophores that grow up from the mycelium, sometimes attaining a length as great as 10 cm. (Fig. 108). If, however, the mycelium is grown in the bottom of a flask filled with beer-wort, where the supply of oxygen is inadequate for the usual growth, then alcoholic fermentation begins and the mycelium divides into single cells, which become separated and resemble those of ordinary yeasts. Thus arises

FIG. 107.—Culture of anaerobes.

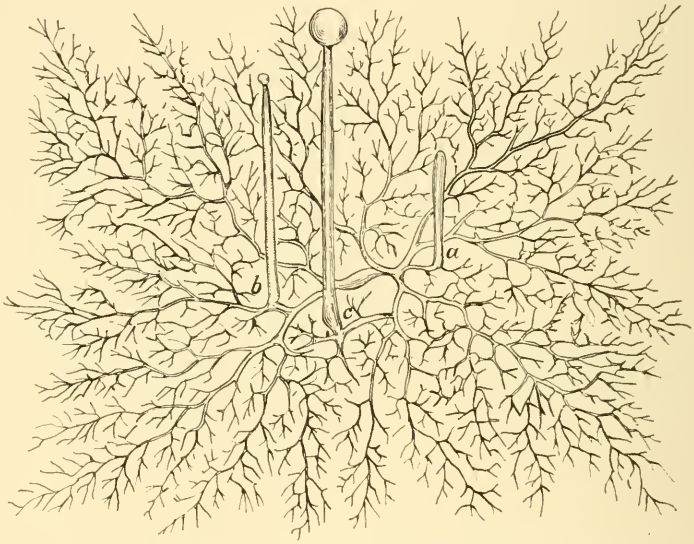


FIG. 108.—*Mucor mucedo*, showing mycelium and sporangiophores.

the so-called mucor yeast (Fig. 109). This example represents an extreme case of the influence of the medium upon the form of organisms.

§3. **Influence of Other Gases on Growth and Configuration.**—Plants grow normally only when the air about them has its usual composition. The carbon dioxide content of the atmosphere is about 0.03 to 0.04 per cent. The investigations of Brown and Escombe[1] and those of Chapin[2] showed, in a quite unexpected way, that an increased carbon dioxide content of the atmosphere was not only not favorable to the growth of certain plants but might even be injurious. An increase in the carbon dioxide content, so that this became 0.2 per cent., resulted in unhealthy plants, which were often very poorly supplied with leaves (Fig. 110). [But improved growth has been secured in many cases by slightly increasing the carbon-dioxide content of the atmosphere.]

[1] **Brown, Horace T.,** and **Escombe, F.,** The influence of varying amounts of carbon dioxide in the air on the photosynthetic process of leaves and on the mode of growth of plants. Proc. Roy. Soc. London **70**: 397–413. 1902.

[2] **Chapin, Paul,** Einfluss der Kohlensäure auf das Wachsthum. Flora **91**: 348–379. 1902.

Neliubov[1] has shown that the form of plants is influenced by the presence of very small amounts of illuminating gas in the air about them, but especially by

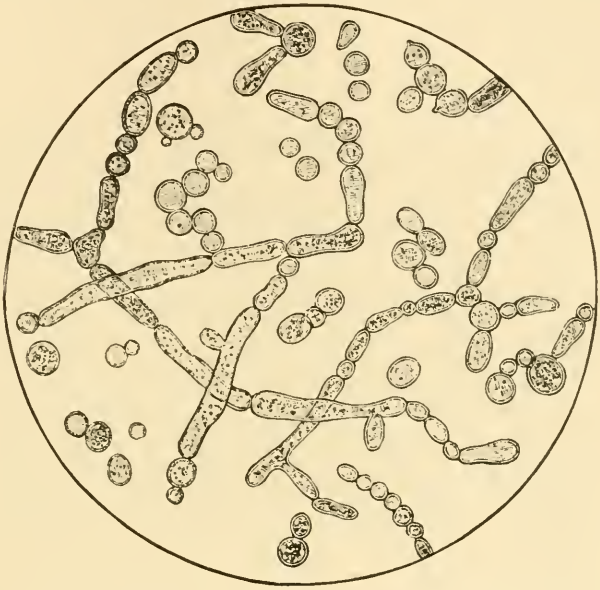


FIG. 109.—Formation of mucor-yeast in an oxygen-free medium.

FIG. 110.—*Impatiens platypetala; A*, in normal atmosphere; *B*, in atmosphere rich in carbon dioxide.

[1] **Neljubow, D.,** Ueber die horizontale Nutation der Stengel von Pisum sativum und einiger anderen Pflanzen. Beih. Bot. Centralbl. **10**: 128–138. 1901. **Idem,** Geotropismus in der Laboratoriumsluft. Ber. Deutsch Bot. Ges. **29**: 97–112. 1911.

ethylene and acetylene, which are present in illuminating gas. The shoots grow erect in an atmosphere without illuminating gas, but when even very minute traces of this gas are present they bend and assume a horizontal position Fig. 111). Many different kinds of gases and vapors are thus injurious to the growth of plants.[1]

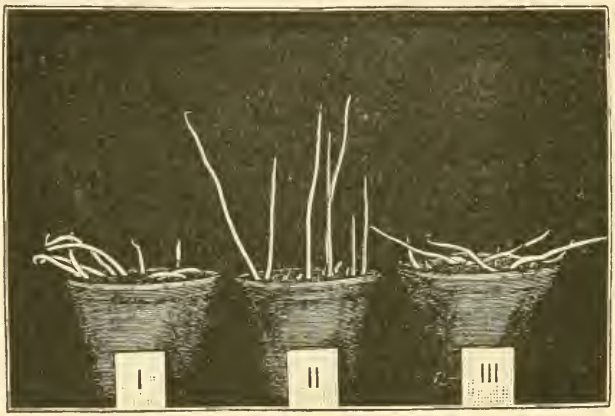
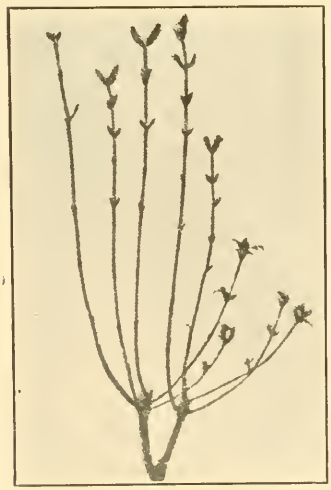


FIG. 111.—Pea seedlings grown in darkness; *I* and *III* in laboratory air containing illuminating gas, *II* in the same air with the poison gas removed. (*After Neliubov.*)



A

B

FIG. 112.—Effect of ether upon the flowering of lilac. All shoots excepting the fifth from the left (as seen in *A*) were treated. The untreated shoot is seen unaltered in *B*, where the others are all in full leaf and flower.

[1] **Haselhoff, Emil,** and **Lindau, G.,** Die Beschädigung der Vegetation durch Rauch; Handbuch zur Erkennung und Beurteilung von Rauchschäden. Leipsig, 1903. [In this connection, see: **Crocker. W.,** and **Knight, L. I.,** Effect of illuminating gas and its constituents on flowering carnations. Plant world 12: 83–88. 1909. **Idem,** Toxicity of smoke. Bot. gaz. 55: 337–371. 1913. **Crocker, W., Knight, L. I.,** and **Rose, R. C.,** A new method of detecting traces of illuminating gas. Science, n.s. 31: 636. 1910.]

On the other hand, some gases have a stimulating effect upon growth. In Johannsen's[1] experiments, bulbs sprouted much more rapidly in an atmosphere containing ethyl ether than in one lacking it. Johannsen recommended treatment with ether as a method for forcing plants. In Fig. 112, *A*, is shown a branch of Syringa (lilac) in November, eight days after treatment with ether vapor; the fifth twig from the left was protected from contact with the gas. In Fig. 112, *B*, the same branch is shown three weeks after treatment, and is in full bloom excepting that the twig originally untreated (here also on the right) still remains leafless.

§4. Influence of Moisture on Growth and Configuration.—The condition of the soil and that of the air, with respect to water, determine the amount of water absorbed and also its rate of movement through the plant. When the atmosphere is saturated with water vapor, transpiration from the leaves is materially lessened and, consequently, the further absorption of water by the roots is similarly decreased. Dry air, on the other hand, accelerates both transpiration and water absorption.

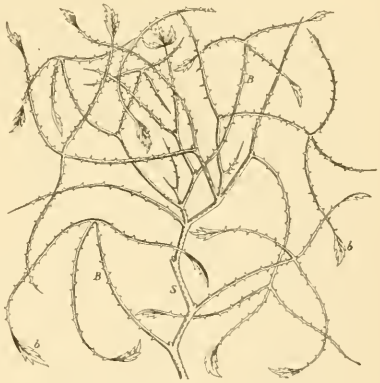


Fig. 113.—A branch of *Rubus squarrosa* (½ natural size). (*After Wiesner.*)

Plants grow luxuriantly only with a plentiful supply of water. Tropical vegetation is exceptionally luxuriant since an abundance of water is here combined with favorable temperature conditions. The virgin forests of the tropics are frequently impenetrable jungles, where plants grow not only on the soil but even on each other (epiphytes). It is quite different with arid regions; the plant world here maintains only a scanty existence. Parallel with the decreased number of plants occurring in arid regions, the individual plant also is smaller in such regions. Plants of moist regions have well-developed foliage, often with very large leaves that have a high water content. Plants in dry climates have relatively small leaf surfaces, so that the loss of water is not so great. Thus the leaves of *Rubus squarrosa* (Fig. 113), which is closely related to the European raspberry (*Rubus idæus*) are very small. Many xerophytes, such as the cacti, have no leaves at all, or they lose them very soon after they are formed. In this case the activities that are usual for leaves occur on the stem. Such plants are furnished with many arrangements that hinder the loss of water. The epidermis is very tough, frequently possesses hairs, and is often covered by wax and other incrustations. Thus *Rochea falcata*, a South African plant, is armed with a siliceous coat of mail. A cross-section of the leaf shows that the small cells of the epidermis are overlaid with a continuous layer of large, bladder-like cells (Fig. 114), the walls of which are richly impregnated with silica. These siliceous cells are filled with water, which is replaced by air only when they become old. As long as

[1] Johannsen, Wilhelm L., Das Aetherverfahren beim Frühtreiben. 2te Aufl. Jena, 1906.

these cells contain water they behave like reservoirs from which the deeper-lying cells of the leaf draw their supply.

The leaves of *Stipa capillata* (Fig. 115) furnish an example of characteristic arrangements that prevent excessive transpiration. Fig. 115, *A′* shows a cross-

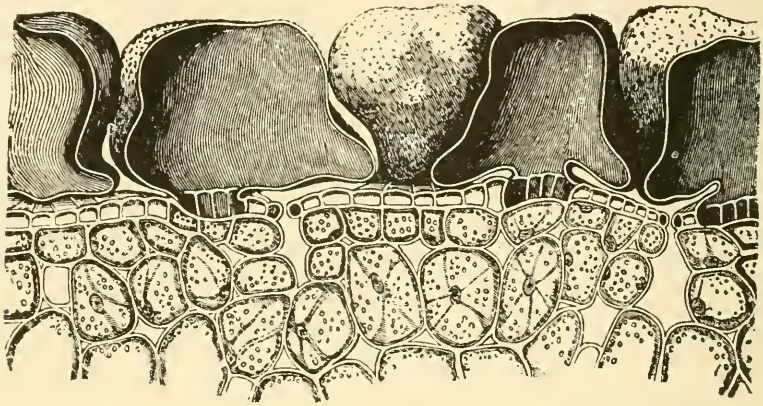


Fig. 114.—Section through leaf of *Rochea falcata*, showing siliceous cells of upper epidermis.

section of a leaf of this plant under normal conditions. When drought begins, however, the stomata not only close but the leaf also rolls and forms a tube (Fig. 115, *A*), so that only one of its surfaces—and indeed the surface that possesses thick cuticle and is quite devoid of stomata—is exposed to the outer

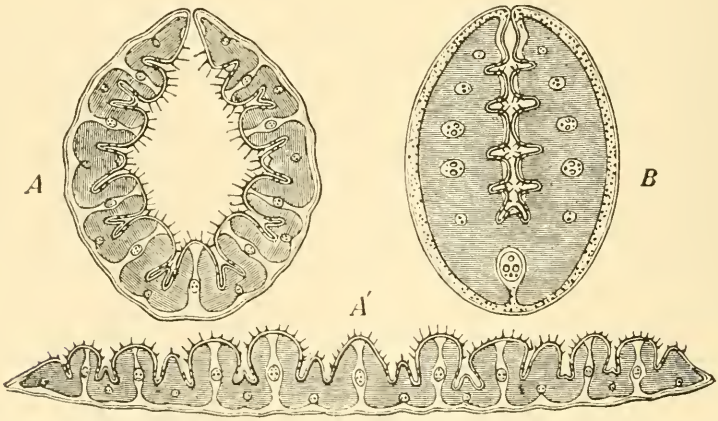


Fig. 115.—Cross-sections through leaves of *Stipa capillata* (*A*, *A′*) and of *Festuca alpestris* (*B*).

air. All the stomata are then on the inner surface of the leaf. Fig. 115, *B*, represents a cross-section of a similarly rolled leaf of *Festuca alpestris*.

Other arrangements are exhibited by *Dischidea rafflesiana*, a climbing plant with two kinds of leaves; some of the leaves have the usual form, but others are

like bags or pouches, with an opening above. A vigorous aerial root arises from the stem at the place of attachment of the bag and grows down into the cavity of the latter. Water collects in the bag when it rains, and this stored water is absorbed by the root and thus transferred to the rest of the plant (Fig. 116).

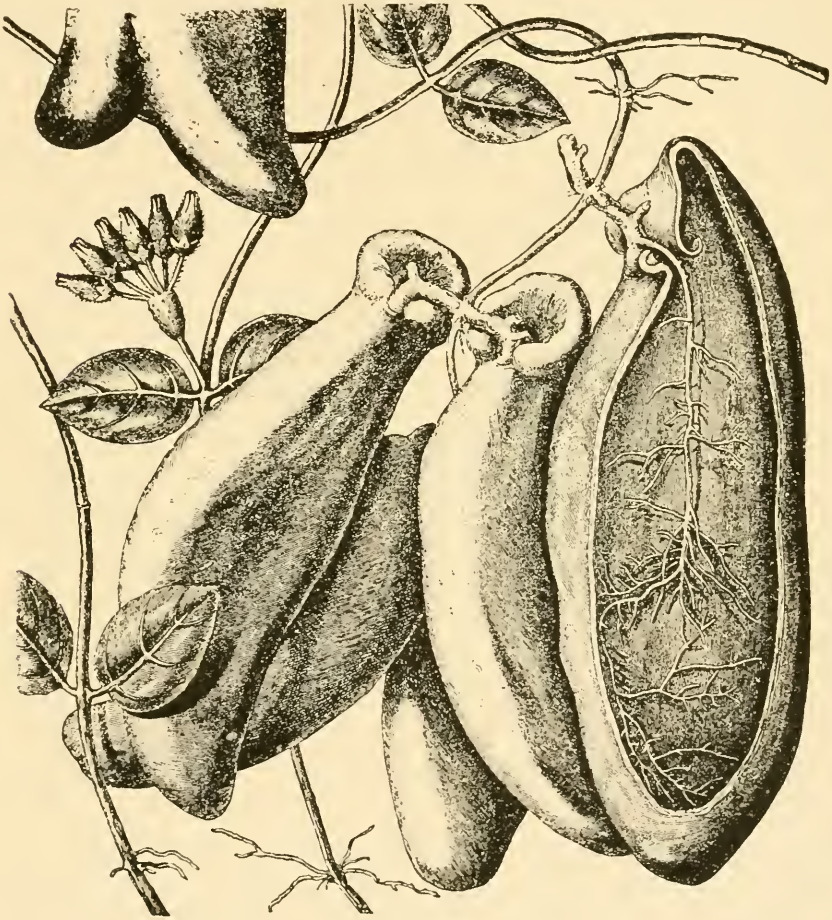


FIG. 116.—*Dischidia rafflesiana*, showing sack-like leaves with aerial roots in the cavity.

Aquatics are likewise distinguished by special structures. Their weak stems are permeated with numerous air passages. The submerged leaves usually have deeply cleft lamina, with filamentous lobes. When such plants develop on land, however, the form of the leaf often becomes remarkedly altered. *Ranunculus fluitans*, for instance, is such an aquatic with filamentous leaves (Fig. 117, 1). When growing on land the aerial leaf has the typical broad lamina (Fig. 117, 2). Several kinds of leaves are frequently found on the same stem.

The flowering specimen of *Bidens beckii* shown in Fig. 118 bears three kinds of leaves. The lower, submerged part of the stem bears the deeply cleft leaves typical of many aquatics. The upper part of the stem, however, which formed above the surface of the water, has simple, nearly entire leaves. In the intermediate region of the stem are found leaves that are intermediate in character.[d] The ordinary arrow-head (*Sagittaria sagittifolia*), which grows in stagnant or slowly-flowing water, has arrow-shaped leaves with long petioles. If the plants are grown entirely under water, then only linear leaves are formed, but if the water level is not very high (Fig. 119), only the completely sub-

FIG. 117.—*Ranunculus fluitans*. 1, water form; 2, land form.

merged leaves remain narrow, while the rest assume the usual arrow-shaped form. There are numerous transition stages between these two forms.

These observations lead to the conclusion that the form of the plant is greatly influenced by the amount of available water. This conclusion is substantiated by direct experiment. If one specimen of an herbaceous annual is grown with rather dry soil and atmospheric conditions, and if another is grown in very moist soil and in a nearly saturated atmosphere, plants of very different structure are developed. The experiment with dry conditions may be conducted

[d] For a discussion of the conditional determination of leaf-form in aquatic plants, see: **McCallum, W. B.**, On the nature of the stimulus causing the change of form and structure in *Proserpinaca palustris*. Bot. gaz. **34**: 93–108. 1902. **MacDougal, D. T.**, The determinative action of environic factors upon Neobeckia aquatica Greene [*Nasturtium lacustre* A. Gray]. Flora **106**: 264–280. 1914.—*Ed.*

by placing the plant under a bell-jar, with a vessel of calcium chloride or concentrated sulphuric acid to reduce the vapor pressure of water. To obtain moist conditions, a sponge saturated with water may be introduced into the bell-jar and the walls of the latter may be moistened. The plant develops long internodes and broad leaf-blades in a moist atmosphere, but short internodes and much smaller leaf-blades prevail under dry conditions. The anatomical characters of the two plants are likewise quite different. Plants that have been cultivated

FIG. 118.—*Bidens beckii*. The lower leaves have formed under water and the upper ones in air.

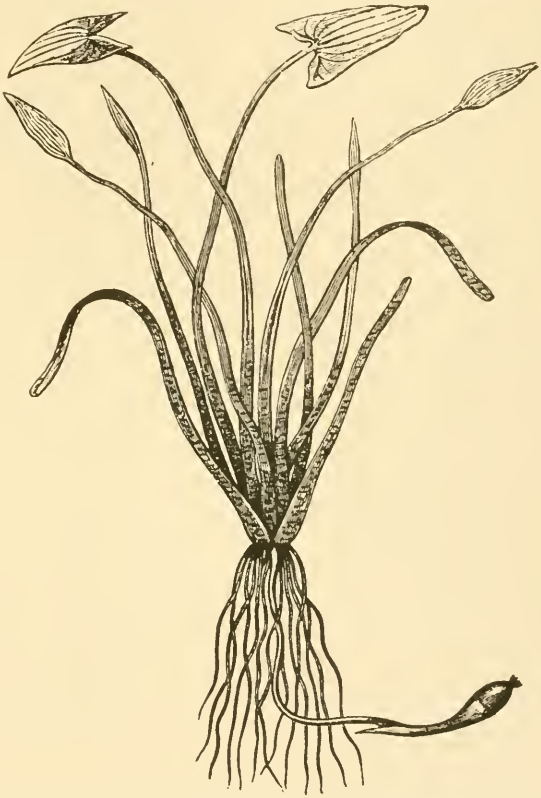


FIG. 119.—*Sagittaria sagittifolia*. Lower, linear leaves formed under water; upper, arrow-shaped leaves formed in air.

with dry soil and dry air have a thick cuticle, well-developed collenchyma, and both bast and wood fibers. Plants grown under moist conditions have thin cuticle and poorly developed woody tissue, and collenchyma and bast fibers are often not formed at all. An experiment with *Tropæolum majus*[1] may serve as an example here. The plants were cultivated under four different sets of conditions, as shown in the table below, which also presents the results of the experiment.

[1] **Kohl**, 1886. [See note 3, p. 135.]

Culture No.	External Conditions		Relative Size of Leaf-blade	Kind of Cuticle	Anatomical Characters	
	Soil	Air			Epidermis	Collenchyma
1	Moist	Moist	5	Thin	Cells tangentially elongated, thin outer walls	None
2	Moist	Dry	4	Thick	Cells radially elongated, thick outer walls	Two adjacent layers well developed
3	Dry	Moist	3	Thin	Cells almost cubical	Poorly developed
4	Dry	Dry	1	Thick	Cells very much elongated radially	Less developed than in 2

The leaves formed by Tropæolum plants growing in moist air and moist soil were thus five times as large as those formed in the driest cultures. In Fig. 120, *D*, is shown a cross-section through the epidermis of a leaf of *Lupinus mutabilis* from a culture in dry air, a corresponding section of a leaf grown in moist air, being shown in Fig. 120, *M*. The differences in the thickness of cell wall and of cuticle are very great. A leaf of the dandelion (*Taraxacum officinale*) grown in a nearly saturated atmosphere is shown in Fig. 121, *A*, similar ones grown under usual conditions being shown in Fig. 121, *B* and *B'*.

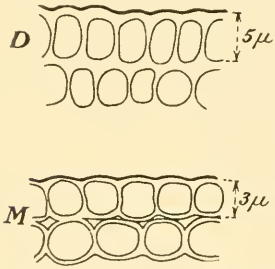


Fig. 120.—Sections of leaf epidermis of *Lupinus mutabilis* *D*, grown in dry; *M*, in moist air.

Plants growing in dry regions often possess thorns, and if such plants are grown in a very moist atmosphere the thorns are generally replaced by short, leafy branches. Two branches of broom (*Genista anglica*) are shown in Fig. 122, one (*C*) grown under normal conditions, the other (*B*) grown in moist air. The difference is so great that they might be taken to be distinct species.

Wiesner[1] has demonstrated that there may be a descending as well as an ascending water stream in plants. The presence of the former may be clearly demonstrated in the following way. A cut branch of grapevine or similar leafy shoot is placed with the youngest, terminal portion of the stem in water, while the rest projects into the air. After some time the part of the stem under water wilts, which is explained by the fact that the actively transpiring leaves remove more water from the terminal portion than it can absorb, in spite of the fact that it is surrounded by water.

Many structural peculiarities of plants may be explained as due to the descending water current. For instance, in many plants a withering of the terminal bud occurs, with the consequent formation of a sympodium. The

[1] Wiesner, J., Der absteigende Wasserstrom und dessen physiologische Bedeutung. Bot. Zeitg. 47: 1–9, 24–29. 1889.

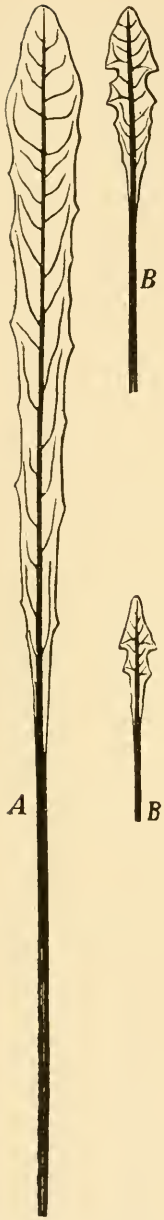


Fig. 121.—Leaves of Taraxacum. *A*, grown in very moist atmosphere (actual length about 60 cm.). *B* and *B'*, grown under usual conditions (actual lengths about 15 and 12 cm., respectively).

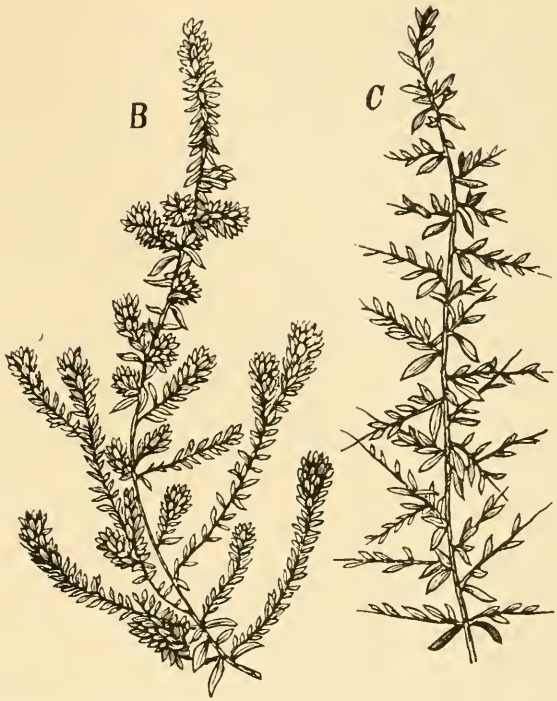


Fig. 122.—Two branches of broom, *Genista anglica*. *C*, grown in dry air; *B*, in moist air.

Fig. 123.—Sempervivum. I, normal; II, grown in moist air; III, grown in darkness.

leaves develop very early in such plants, so that active leaves are formed immediately beneath the terminal growing-point. These leaves withdraw water, by transpiration, from the terminal bud and thus cause its destruction. If such plants are cultivated in an atmosphere nearly saturated with water vapor, the terminal bud is protected from destruction and the stem develops with monopodial branching. Various plants that usually have short internodes, such, for instance, as *Bellis perennis*, *Capsella bursa-pastoris*, and Sempervivum when cultivated in a saturated atmosphere, develop a stem with leaves arranged spirally (Fig. 123). In these cases the reduction of the primary stem occurring under usual conditions is due to a deficiency of water; the rosette of leaves forms rapidly and transpires very actively, thus depriving the terminal bud of adequate water supply.

All these observations and experiments show that the same species may be very definitely modified, in external form as well as in internal structure, by variations in the moisture condition of soil and air, and that the changes thus produced are very striking. The question now arises: Why does the amount of water absorbed by the plant have so great an influence upon its formal development?

Turgidity, as is well known, is a condition essential to growth. The more water is contained in the plant, the more its cells can be stretched from within. Enlargement is terminated when water ceases to enter the cell. Wortmann found, in experiments with *Lepidium sativum*, that root-hairs are very long and thin when grown in water, while they remain short and their cell walls are much thickened when they are grown in sugar solutions. The cellulose that produced the increased expanse of cell wall in the first case, produced thickening in the second case. The same thing occurs when the water supply is not sufficient for the usual growth of the plant, small cells with thick cell walls being formed in this case also.

Substances dissolved in water influence the entrance of the solvent into the cells, not only by their osmotic activity, but also by changes that they may produce in the protoplasmic membrane, as was shown by the investigations of Ritter.[1] This author found that both organic and inorganic acids produce striking structural changes in the hyphæ of some of the lower fungi, especially the Mucorinæ. Giant cells are formed which may be thirty or forty times as large as are the ordinary hyphal cells. The giant cells of *Mucor spinosus* are typical of these; they are formed when the spores are allowed to germinate in a nutrient medium containing citric, tartaric, or malic acid.

This phenomenon is due, at any rate, to a change in the osmotic properties of the plasma membrane under the influence of acids. Such a conclusion is supported by the later work of Czapek,[2] who has furnished direct evidence favoring the hypothesis that acids may greatly increase the permeability of the plasma membrane, thus facilitating the outward diffusion of substances dissolved in the cell sap.

[1] Ritter, G., Ueber Kugelhefe und Riesenzellen bei einigen Mucoraceen. Ber. Deutsch. Bot. Ges. 25: 255–266. 1907.

[2] Czapek, F., Versuche über Exosmose aus Pflanzenzellen. Ber. Deutsch. Bot. Ges. 28: 159–169. 1910.

Further examples of the effect of dissolved substances upon the permeability of protoplasm may be found in the work of Demoore and Szücs. Demoore[1] found that the addition of peptone to a very weak solution of sodium chloride, which itself had no injurious effect upon the cell, greatly increased the permeability of the protoplasm. Sodium citrate neutralized this action of the peptone. Szücs[2] showed that the addition of an electrolyte retards the entrance of basic aniline dyes into the cell.

Alterations in the turgidity of the cell, due to changes in the amounts of water and of dissolved substances in the surrounding medium, are among the causes that bring about changes of form in plants. The amount of water vapor in the surrounding air influences the rate of plant transpiration, and the more water is lost by transpiration, the more is absorbed from the soil, if the supply is adequate. But plants absorb, along with water, the essential ash-constituents and upon the latter depend, in turn, the formation and migration of various organic substances. That the amount of water absorbed determines not only the external form and the internal structure of the plant but also its chemical composition, may be seen from the experiments of Schlösing.[3] He cultivated tobacco plants under glass bell-jars and also in the open air. The dry weight produced in the moist atmosphere in four weeks was 40 g., while that produced in ordinary air in six weeks was only 29.4 g. The leaves of the moist culture formed and accumulated more non-aqueous material. But this material of the moist culture contained less ash; the ash content, in percentage of the total dry weight, was 13 per cent. for the moist culture and 21.8 per cent. for the culture in ordinary air.

These analyses also show that the leaves of the moist culture of this experiment differed in other ways from those grown under usual conditions. The modified rate of transpiration affected also the formation of various organic compounds. The following table shows the amounts of various substances found by Schlösing in the leaves of his plants grown under the two sets of conditions, the numbers representing percentages, on the basis of the dry weight of the leaves.

	Moist Conditions	Usual Conditions
Urea	4.00	5.02
Nicotin	1.32	2.14
Other nitrogenous compounds	17.40	18.00
Oxalic acid	0.24	6.66
Citric acid	1.91	2.79
Malic acid	4.68	9.48
Pectic acid	1.70	4.36
Cellulose	5.36	8.67
Starch	19.30	1.00

[1] **Demoore, J.,** Influence du citrate de soude sur les échanges cellulaires. Bull. Soc. Roy. Sci. Méd. et Nat. Bruxelles, No. 4, p. 79–81. 1909. [Rev. by **Micheels** in : Bot. Centralbl. 116 : 166. 1911.]

[2] **Szücs, Josef,** Studien über Protoplasmapermeabilität. Ueber die Aufnahme der Anilinfarben durch die lebende Zelle und ihre Hemmung durch Elektrolyte. Sitzungsber. (math. naturw. Kl.) K. Akad. Wiss. Wien. 119^{I} : 737–773. 1910.

[3] **Schlösing,** 1869. [See note 1, p. 147.] [But for another study on tobacco, giving quite the opposite conclusion, see: **Hasselbring,** 1914. (See note *w*, p. 148.)—*Ed.*]

The very large amount of starch found in the leaves grown in moist air is specially noteworthy, as is also the observation that this high starch content is concomitant with relatively low amounts of the other substances here considered. It is supposed that the carbohydrates formed in the leaves are combined, in other regions of the plant, with elements derived from the soil solution; in this case there was a deficiency of these elements in the plants grown in moist air, owing to their low transpiration rate, so that much of the starch was retained in the leaves. This great accumulation of starch is probably one of the causes for the relatively large size of leaves grown in a moist atmosphere. Differences in inorganic salt content therefore constitute a second cause for the differences in plant form produced by differences in the water conditions of the external environment.

Plant growth and development are markedly influenced by the concentration of dissolved mineral salts in the surrounding medium, as has been known for a long time, from studies of plant cultures in solutions of different concentrations. Plants grown in weak solutions resemble those of moist regions while those grown in very strong solutions have a markedly xerophytic appearance.[1] It is immaterial, therefore, whether the plant receives excessive amounts of the minerals through high rates of transpiration or through culture in concentrated solutions, the result being the same in both cases: namely, the formation of short internodes, thick cell walls, etc., with generally marked tissue differentiation.[e]

Many strand plants have xerophytic characteristics in spite of the moist surroundings in which they grow. Schimper[2] noted this fact and "explained" it teleologically by supposing that these plants, growing in sand that is frequently saturated with a concentrated salt solution—from the ebb and flow of the tide

[1] **Nobbe, F.,** and **Siegert, T.,** Beiträge zur Pflanzencultur in wässerigen Nahrstofflösungen. I. Ueber die Concentration der Nahrstofflösungen. Landw. Versuchsst. **6**: 19–45. 1864. [For a thorough review of the literature of water-cultures, see: **Tottingham,** 1914. (See note *d*, p. 84.)]

[2] **Schimper, A. F. W.,** Ueber Schutzmittel des Laubes gegen Transpiration, besonders in der Flora Java's. Sitzungsber. K. Preuss. Akad. Wiss. Berlin **1890**: 1045–1062. 1890.

[e] Whether these responses have any relation to the supply of mineral salts is at least questionable. The phenomena here dealt with in a very cursory way are exceedingly complex and cannot be generally and satisfactorily explained along the lines followed by the author. The water content of the tissues appears, in itself, to act as the main control in such cases as are here brought forward. It ought to be remarked that this water content of the plant, or of any tissue, is a function of the relation that has previously obtained between the rates of water entrance and of water exit. Concentrated solutions about the roots retard water entrance in much the same way as does a soil of low moisture content. The last sentence in the text might be reasonably replaced by the following one: It is immaterial, therefore, whether the water content of the plant becomes low through high rates of water loss or through low rates of water intake.—For discussions of some of the considerations that are not clearly set forth in the text but are quite necessary in dealing with this general subject of plant water relations, see: **Livingston, B. E.,** and **Hawkins, Lon A.,** The water-relation between plant and soil. Carnegie Inst. Wash. Pub. **204**: 3–48. 1915. **Pulling, H. E.,** and **Livingston, B. E.,** The water-supplying power of the soil as indicated by osmometers. *Ibid.* **204**: 49–84. 1915. These papers furnish numerous other references to the literature.—*Ed.*

—develop a number of structural adaptions *in order* to retard transpiration and so prevent too great an accumulation of mineral salts.[f]

Plants of the far north frequently have xerophytic characters also, even though they grow in very wet soil. Under these conditions they may suffer from a deficiency of water,[1] for the entrance of water into the roots is dependent upon certain temperature conditions; water absorption is slow when the soil is cold, and if, at the same time, the atmospheric conditions produce high rates of transpiration, then wilting may very easily occur, even though the roots are surrounded with water. A heavy cuticle prevents the external conditions from raising the rate of transpiration as much as they would if the cuticle were thinner.[g]

[1] **Kihlmann, A. Osw.**, Pflanzenbiologische Studien aus Russisch-Lappland. Ein Beitrag zur Kenntnis der regionalen Gliederung an der polaren Waldgrenze. Helsingfors, 1890.

[f] Of course this is not an explanation, and it has no bearing on the problem in hand. Plants do not produce peculiar structures "in order to retard transpiration" or for any other purpose; the peculiar structures result from the interaction of preëxisting conditions, and the *effect* of the presence of these structures, after they are produced, is to retard water loss. For a working hypothesis, it may be supposed that the high salt content of the soil retards water intake in the case of these strand plants (either osmotically or by a chemical influence upon the root protoplasm, such as rendering this only slowly permeable to water), and that the open exposure of such plants makes the rate of water loss (transpiration) relatively high, so that the water content of the tissues is maintained comparatively low.—*Ed.*

[g] The heavy cuticle of such plants may result from low water content of the tissues (see note *f*, just preceding).—It appears that one main reason for the dominance of plants with foliar structures that retard transpiration, in bogs, and perhaps generally in the far north, is the presence of toxic materials in the soil. (See note *k*, p. 101.)

This whole discussion, as given in the text, is rendered unsatisfactory by the confusion of two entirely distinct problems, one physiological and the other in the realm of distributional ecology. From the standpoint of physiology, we should seek the conditions (internal and external) that make one plant produce xerophilous structures, etc., while another does not. This involves experimental problems like that dealt with by Schlösing and by Hasselbring (page 271), and like that considered by the author in reference to the experiments of Nobbe and Siegert (page 272). Without adequate measurement of the effective conditions that obtain, a knowledge of these relations cannot be achieved by ordinary field observations, no matter how thoroughly such observations may be subjected to subsequent attempts at interpretation.

From the standpoint of distributional ecology, on the other hand, we desire to know, first, what physiological types of plants occur, and are dominant, in different habitats and geographical regions. As an example of this sort of knowledge we have the observed fact that thick foliar cuticle is of dominant occurrence on the plants of bogs and of the far north. The ecological interpretation of this observation does not have anything at all to do with the physiological question as to what may be the necessary conditions for the *production* of thick cuticle, but it does deal with the question as to what kinds of environmental complexes may prevent the development of *plant forms* that do not produce such cuticle, at the same time allowing forms that *do* produce thick cuticle to dominate. Given a number of plants, some with and some without xerophilous foliar structures (no matter by what sets of conditions these structures may have been produced or inhibited in the different cases), we observe that bog habitats are characterized by the dominance of plants of the first class, and we suppose that plants of the second class (without xerophilous foliar structures) are generally unable to thrive in such habitats. The question then emerges, as to what are the peculiar environmental conditions that so generally prevent the growth of the non-xerophilous forms. The generalized

Unequal amounts of moisture on the two opposite sides of a plant organ also exert an influence upon growth. If seeds germinate in a sieve filled with sawdust and suspended so that its bottom is at an angle of 45 degrees from the horizontal (Fig. 124), the primary roots soon penetrate through the openings in the bottom, but they grow no farther in the vertical direction. They bend laterally toward the bottom of the sieve and grow downward along its outer surface, to which they become closely appressed. This bending of plant organs toward water, or away from the drier side, is called positive *hydrotropism.*

§5. Dependence of Growth and Configuration upon Light.[1]—Light exerts a marked influence upon the rate of plant growth as well as upon the formation

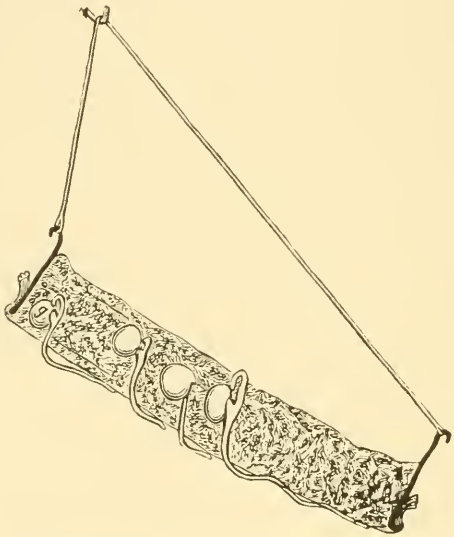


Fig. 124.—Experiment showing positive hydrotropism of roots.

answer to this question seems to be, *soil conditions that hinder water absorption.* Toxic substances appear to do this by poisoning the roots, so that these organs possess but a limited power to take up water, in spite of the presence of a plentiful supply of water in the soil. Low soil temperature (as in subarctic regions) may hinder water absorption from wet soils in somewhat the same way. These considerations may furnish at least a partial explanation of the fact (if it be a fact) that plant forms without special foliar structures that retard water loss are generally unable to thrive in bogs and in the far north.

The ecological question just touched upon is one with which physiology, as such, need not be concerned, and distributional and physiological problems ought not to be so commonly confused as is now the case in botanical literature. Physiology enquires how the plant comes to be what it is, and how it operates as a machine. Its explanations have to deal with migrations and transformations of matter and energy. Distributional ecology, on the other hand, enquires what are the characteristics of any plant form and of any given set of environmental conditions, by virtue of which the given habitat can or cannot support the plant form considered, or by virtue of which the plant form can or cannot thrive in the given habitat. How the conditions of the habitat came to be what they are, involves questions of climatology, physiography, soil science, etc.; why the plant has the internal characteristics that it has, involves questions of physiology.—*Ed.*

[1] **Wiesner, J.,** Der. Lichtgenuss der Pflanzen. Leipzig, 1907 **Idem,** same title. Verhandl. Ges. Deutsch. Naturforscher u. Aerzte **81**: 66–86. 1909.

of individual organs. The most common phenomenon to be noted in this connection is the daily periodicity of growth. Plants grow more slowly by day than by night, so that it appears that light exerts a retarding influence upon growth.[1] The growth maximum occurs in the early morning hours and the minimum occurs in the evening. The curve $3z$ of Fig. 125 shows the diurnal march of the rate of plant growth, which is seen to increase gradually from about 6 p.m. to about 6 a.m., after which it gradually decreases, from morning until evening. The accelerated growth of the night hours occurs in spite of the lower night temperature, as may be seen from the figure just mentioned, where the curve $t°$ represents the diurnal march of temperature corresponding to that of growth. This periodicity is mainly dependent upon light, although it continues to be manifest—but with less regularity—when the plant is kept continuously

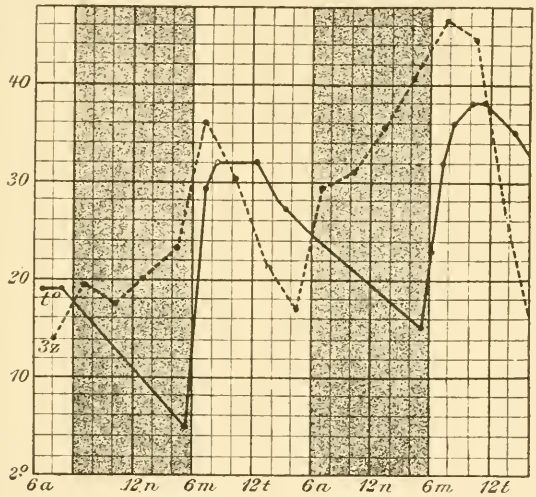


FIG. 125.—Graph showing daily periodicity of growth, the broken line $3z$. The full ($t°$) is corresponding graph of temperature. (*After Sachs.*)

in darkness. The latter fact has been explained as an induced rhythm; the ancestors of the present plants have been exposed for countless generations to the diurnal alteration of light and darkness, and the periodicity of growth appears to have become a habit (due to internal conditions), which is more or less markedly inherited.

One-sided illumination brings about a bending of plant organs, this response being termed *phototropism* or *heliotropism*.[2] When an organ bends toward the more brightly lighted side it is said to be positively phototropic; when it bends away from the more intense light it is negatively phototropic. Positive phototropism is very common among plants and is usually observed when growing stems are subjected to one-sided illumination.

[1] **Baranetzky, J.,** Die tägliche Periodizität im Längenwachstum der Stengel. (Mém. Acad. Imp. Sci. St.-Pétersbourg, VII, 27^2: 1–91. 1897. **Godlewski, Emil,** Studyja nad wzrostem roslin. Krakau, 1891.*

[2] **Wiesner, Juluis,** Die heliotropischen Erscheinungen im Pflanzenreiche. Eine physiologische Monographie. I Theil. Denksch. K. Akad. Wiss. Wien 39^I: 143–209. 1879. **Idem,** same title. II Theil. *Ibid.* 43^I: 1–92. 1882. **Idem,** Das Bewegungsvermögen der Pflanzen. Wien, 1881. P. 37–84.

Among plants that are especially sensitive to these differences in light intensity, on the two opposite sides, may be mentioned *Vicia sativa*. If etiolated seedlings of this plant are placed between two sources of light differing so slightly that the difference cannot be detected by ordinary photometric methods, the seedlings always bend promptly toward the source of the more intense light. Phototropic bending is often difficult to observe in plants growing in sunny places in the open, such as *Cichorium intybus, Verbena officinalis, Sisymbrium strictissimum, Achillæa millefolium* (yarrow). If such plants are grown in weaker light, however, the light reaction becomes apparent. The stems of Dipsacus (teasel) and Equisetum are but slightly phototropic and those of

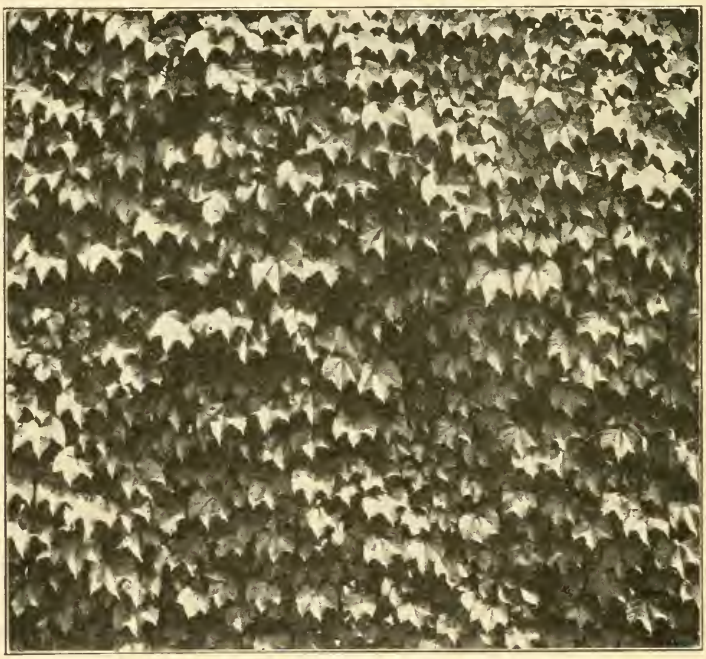


FIG. 126.—Leaf-mosaic of Boston ivy. (*From Gager.*)

Verbascum thapsus (mullein) and *V. phlomoides* do not exhibit phototropism at all.

Phototropic responses occur very commonly in leaves, these organs tending to assume such positions that they do not shade one another. Observed from above, such an arrangement of leaves appears like a mosaic, as in the case of the ivy leaves shown in Fig. 126. In this case, the lobes of one leaf approximately fill the indentations of others, so that a closely fitting arrangement results.

Many leaves bend so as to place the blades at right angles to the direction of strongest illumination (Fig. 127). Shortly after sunrise the upper surfaces of these leaves are inclined toward the east, at midday the blades take a nearly horizontal position, and in the evening they are turned toward the west. In

all these cases the upper surface of the leaf-blade becomes so oriented that it is perpendicular to the direction of the impinging rays. Even if such a plant is in-

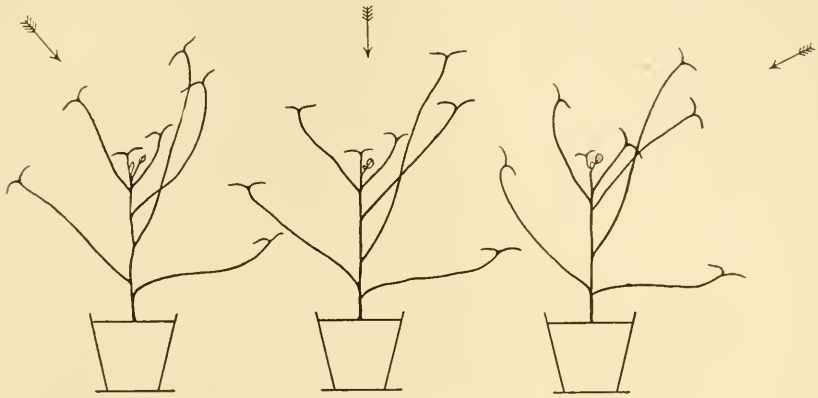


FIG. 127.—Diagrams showing phototropic movements of leaves, with reference to the direction of impinging light, this direction shown by the arrows.

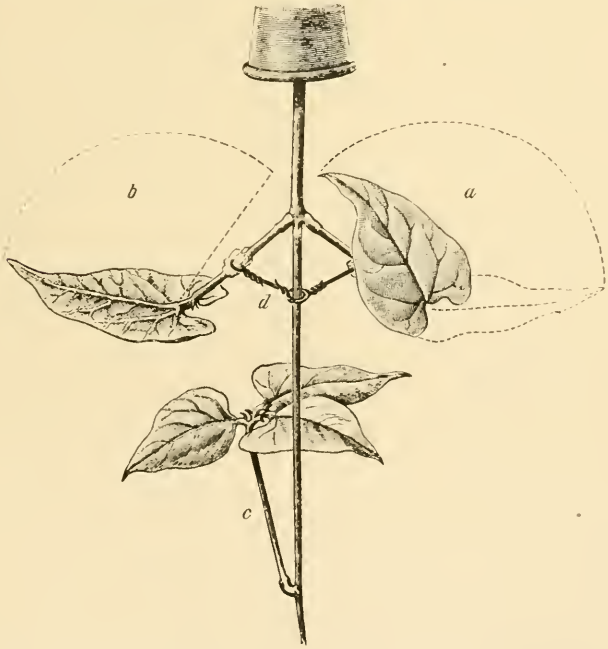


FIG. 128.—Inverted Phaseolus plant. Two petioles are fastened with wire so as to hold them in their normal position. Leaf-blade *b* is represented as in its normal position, while *a* has become re-oriented after the plant was inverted. Leaf *c* has responded by a torsion of the petiole as well by a bending. (*After Pfeffer.*)

verted (Fig. 128) the leaves bend in such a way as to direct their normally upper surfaces toward the source of strongest illumination,[1] the movement being

[1] **Vöchting, Hermann,** Ueber die Lichtstellung der Laubblätter. Bot. Zeitg. 46: 501–514, 517–527, 533–541, 549–560. 1888.

brought about by either a bending or a twisting of the petiole, frequently by both of these processes together. If the plant is inverted and lighted only from below, then the leaves react so as to maintain their normally upper surfaces directed downward, toward the source of illumination.

FIG. 129.—Compass-plant, *Sylphium laciniatum* as seen from the east or west (left), and as seen from the north or south (right). (*After Stahl.*)

What has been stated above concerning the phototropism of leaves holds for most plants, but there are a few exceptions. The leaves of some plants growing in hot regions do not find their position of phototropic equilibrium when the leaf-blade is perpendicular to the direction of the impinging light, but they bend so as to make the blade assume an acute angle to the line of the light rays. Finally, there are so-called compass-plants,[1] which more or less regularly bring their leaves into a position so that the two faces of the blade face east and west, the leaf-tips pointing obliquely upward and alternately north and south (Fig. 129). This arrangement results in the so-called profile position of the leaves at midday, at which time the leaf surfaces are parallel to the direction of the direct rays of sunlight, an orientation that tends to render them less liable to excessive heating. Such reactions to light are more or less perfectly exhibited in *Sylphium lacineatum*, *Lactuca scariola* (wild lettuce), and others.

Many flowers also exhibit the phototropic response. Several species of Tragopogon furnish examples of flower-heads that bend toward the sun. Before sunrise the flower-heads all bend toward the east, though they are still closed. They open as soon as the sun rises. In the morning a meadow of blossoming Tragopogon appears all bright with flowers when viewed from the east, but looks uniformly green when seen from the west; in the latter case only the green involucres of the flower-heads are seen. During the day the flowers change their position as the sun advances

[1] Stahl, E., Ueber sogenannte Compasspflanzen. Jenaische Zeitsch. Naturwiss. 15: 381–389. 1881.

across the sky, and in the evening they all face the west. They close about sunset and then become erect on their stalks, remaining so until morning, when movement begins anew. This movement can be stopped by very intense light. In Fig. 130 are shown closed and open flower-heads of Hieracium, a plant closely related to Tragopogon and showing the same responses.

Phototropic bending occurs also in non-green plants—in moulds, for example. If fresh horse dung is placed in a closed chamber with a small glass window, a dense growth of Pilobolus soon develops and the sporangiophores all bend toward the window. The sporangia, containing the ripe spores, are thrown with considerable force, well-aimed at the glass window, to which they adhere (Fig. 131).[1]

Negative photropism is not very common, but occurs with many tendrils and aerial roots. Weisner[2] studied the aerial roots

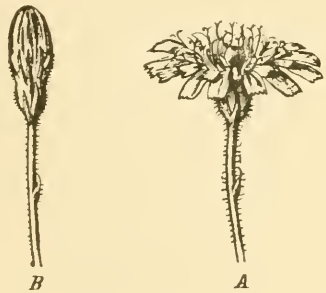


FIG. 130.

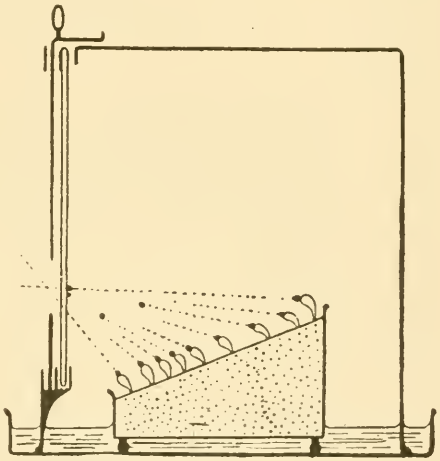


FIG. 131.

FIG. 130.—Flower of *Hieracium pilosella*. *A*, open, as by day; *B*, closed, as by night.

FIG. 131.—Diagram showing phototropic response of Pilobolus. The culture is in a chamber and receives light only through small window at left. Spore-masses are discharged toward the window.

of sixty-one different plant forms and found that the negative phototropic response was very marked in twenty-seven species and was not so marked in twenty-four species, while six species showed but little sensitiveness to light and the remaining four were not sensitive at all. This phenomenon does not occur commonly in ordinary subterranean roots, but if mustard seedlings (*Sinapis alba*) are grown in water-culture it is easy to demonstrate both positive phototropism of the shoots and negative phototropism of the roots.

Phototropic bending results from unequal growth on the two sides of the organ in which this bending occurs, and the response takes place only in the enlarging region. The degree of bending, or its rate, depends upon light intensity. Light of medium intensity produces the most pronounced bending, and the response is less marked both with higher and with lower intensities. The phototropic response is slight when the light intensity is low, increases to a maximum with medium light intensities, and becomes less when the light in-

[1] For an excellent study of the light reaction of Pilobolus, see: **Parr, Rosalie,** The response of Pilobolus to light. Ann. bot. **32**: 177-205. 1918.

[2] [**Wiesner,** 1879, 1882. [See note 2, p. 275.]

tensity is still further increased. It is due to unequal illumination of the two sides of the sensitive region of the bending organ, and the difference in illumination between the two sides is of course generally greatest with medium intensities of the light impinging on them. When the light upon one side of an organ is very strong the tissues are penetrated and the cells on the opposite side receive nearly as much illumination as do those on the directly illuminated side. It is for this reason that phototropic bending is not frequent in plants growing in intense sunshine, and this explains the retardation of the phototropic movement in Tragopogon when exposed to intense light.[h]

The various wave-lengths of sunlight do not all have the same phototropic influence upon plants, as is shown by the graphs of Fig. 132. In this figure the letters at the base represent the positions of the Fraunhofer lines in the solar spectrum, A, B, C, etc. The curve *XY* represents the comparative *growth*

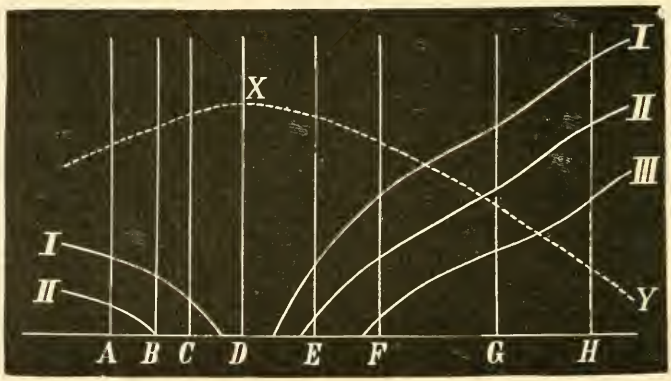


Fig. 132.—Graphs representing rates of growth and phototropic sensitiveness of plants in various wave-lengths of sunlight.

rates of sunflower seedlings in the different regions of the spectrum, this rate being highest at *X* and lowest at *Y*. Curve I represents the *phototropic sensitiveness* of vetch seedlings, curve II that of cress seedlings, and curve III that of etiolated willow shoots.

In yellow light, about the D-line, no phototropic response is apparent. With longer or shorter wave-lengths phototropism becomes evident, and the sensitiveness of the plants becomes greater as the wave-length increases or diminishes. The rays of the shorter wave-lengths, in the right half of the spectrum, are the most effective to produce bending in all three cases, and etiolated willow shoots fail to show any response to the long wave-lengths of the red region. Thus, of the visible spectrum, violet light is most effective to produce phototropic bending.

Light retards plant elongation, as is clear from the daily periodicity of growth, but this retardation differs in amount with different wave-lengths.

[h] Also, with strong illumination the light received by reflection from the sky, from surrounding objects, etc., is comparatively intense.—*Ed.*

The curve XY of Fig. 132 shows that the greatest retardation occurs with violet and ultra-violet light, this effect decreasing with longer wave-lengths until it is minimal in the yellow region, about the D-line. Beyond this region, with still longer wave-lengths, the retarding effect again increases. These facts furnish an explanation of the differences between the phototropic responses brought about by different qualities of light. The greater is the growth-retarding effect of any given quality of light, the stronger is its phototropic influence. Other conditions remaining unchanged, the most pronounced phototropic influence is exerted by light that impinges perpendicularly to the surface of the sensitive plant organ.

Phototropism is of great ecological significance. Positive phototropic responses bring the plant and its parts into the most favorable conditions of illumination, and the negative responses of tendrils and aerial roots take these organs out of the sunshine into the vicinity of surfaces to which they can become attached, such as the surfaces of fences, walls, tree-trunks, etc.

It has recently been shown that many plants possess special structures that are supposed to act as organs of light-perception.[1] For example, the epidermal cells of the leaves of *Campanula persicifolia* are characterized by condensing lenses in their outer walls, these thickenings being impregnated with silicic acid. These lens-like structures are somewhat similar to the lenses of animal eyes.

It has been seen that temporary absence of light (as during the night hours) and one-sided illumination, which brings about phototropic responses, are both markedly effective in determining the rate of growth and the formal development of plants, and it is now to be added that prolonged absence of light exerts an even more pronounced influence. Plants grown in darkness are very different from those exposed to the ordinary succession of day and night. Such plants are said to be *etiolated;* they differ greatly in form but are primarily characterized by having yellow leaves and white stems.[2]

In plants that do not produce stems in darkness (such as wheat), the dark-grown leaves are longer and narrower than are leaves grown in light. In such plants the leaf surface is generally greater when they are etiolated than when they are grown in light. In plants that form stems in darkness, the internodes are much longer in darkness than in light and the leaves remain rudimentary in darkness. In this class belong the pea (*Pisum sativum*), the Windsor bean (*Vicia faba*), millet (*Panicum miliaceum*), the potato (*Solanum tuberosum*), etc. The scarlet-runner bean (*Phaseolus multiflorus*) is also one of this class; it is

[1] **Haberlandt: G.**, Die Lichtsinnesorgane der Laubblätter. Leipzig, 1905.

[2] In this connection, see: **Sachs, Julius,** Ueber den Einfluss des Tageslichts auf Neubildung und Entfaltung verschiedener Pflanzenorgane. Bot. Zeitg. **21**: (Beilage; separately paged, 1–30). 1863. **Batalin, A.,** On the influence of light upon the structural development of plants. [Russian.] Dissertation. St. Petersburg, 1872. [Latest German paper located is the following: **Batalin, A.,** Ueber die Wirkung des Lichtes auf die Entwicklung der Blätter. Bot. Zeitg. **29**: 669–686. 1871. For an account of a large amount of experimentation upon the morphogenic influence of light see: **MacDougal, D. T.,** The influence of light and darkness upon growth and development. Mem. New York Bot. Garden, v. 2. XIII + 319 p. New York, 1903. On the influence of different lengths of alternating periods of light and darkness, see: **Garner, W. W.,** and **Allard, H. A.** Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agric. Res. **18**: 553–606. 1920.

shown in the etiolated and in the usual condition in Fig. 133.[i] Most etiolated stems fail to develop lateral branches, but the etiolated potato sprout is an exception to this rule. It has much-elongated internodes and rudimentary leaves, but it bears small lateral branches (Fig. 134).

Many plants that develop only very short stems in light, with leaves in rosettes, like *Bellis perennis* and Sempervivum (Fig. 123, page 269), form elongated stems in darkness, with spirally arranged leaves.

Another group of plants that do not produce longer internodes in darkness than in light includes those in which, under normal conditions, the leaves are much retarded in their development and the young internodes quickly become greatly elongated. Such forms, among which belong the hop (*Humulus lupulus*)

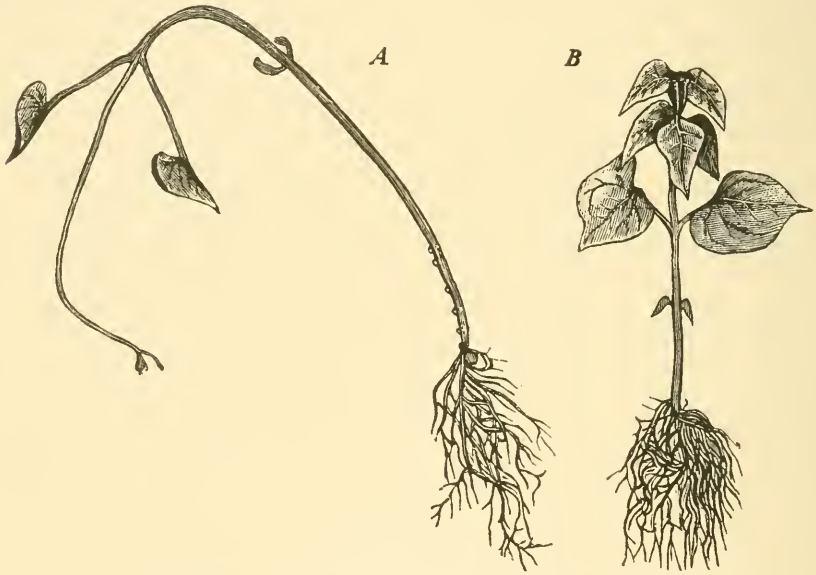


FIG. 133.—Seedlings of scarlet-runner bean. *A*, grown in darkness; *B*, grown in light.

and *Polygonum dumetorum*, when grown with the alternating light and darkness of day and night, develop full-grown leaves only on the older internodes, which have ceased to elongate. Thus, the younger, elongating portion of the plant appears very much as if it were etiolated, and no marked difference in the

[i] In such twining plants as the scarlet-runner bean the manner of growth of the younger portion of the shoot changes as they become older and the long internodes and small leaves of etiolated plants are produced, even in the presence of light. Thus, if the plant shown in Fig. 133, *B*, continued to grow in light it would soon become terminated by a long, slender shoot such as is shown in *A* of this figure. This kind of etiolation, generally shown by the younger portions of the stems of twiners, occurs in light, but it is similar to the etiolation of other plants (or of the same plant in its early stages of development) that is brought about by absence of light. As the light-grown shoot becomes older its leaves finally expand, however. This matter receives attention in the text, just below, where Humulus and *Polygonum dumetorum* serve as examples. See also page 311.—*Ed.*

form of this region is brought about when these plants are grown in continuous darkness.

Phyllocactus, which produces flat, leaf-like stems and branches under usual conditions, forms slender, cylindrical internodes in continuous darkness.[1]

When darkness produces etiolation the anatomical structure of etiolated plants is also different from that of the same forms grown in light; the dark-grown individuals are characterized by exceptionally well-developed thin-walled parenchyma, by exceptionally thin cuticle, by small size and number of the vascular bundles, and by a pronounced retardation in the formation of mechanical tissue.

Experiments with colored light-screens show that plants assume their usual forms only when they receive blue and violet light. When grown in light of other colors [that is, with the intensity of the blue and violet rays very greatly diminished as compared to their intensity in sunlight], etiolation becomes manifest.[2] The curve *XY*, Fig. 132, shows how greatly growth is retarded by blue and violet light.

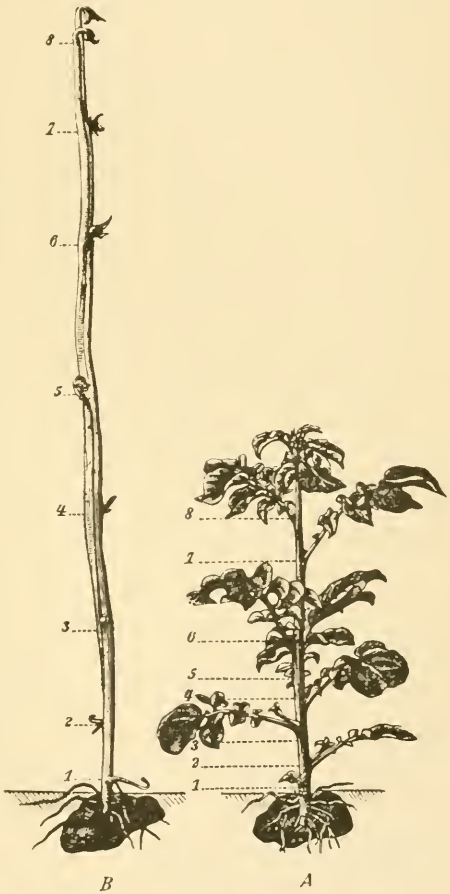


FIG. 134.—Potato sprouts grown in light (*A*) and in darkness (*B*). (*After Pfeffer.*)

The fact that photosynthesis cannot occur in darkness was formerly supposed to explain the phenomena of etiolation, but the experiments with colored lights just mentioned show clearly that the photosynthetic process has practically no direct influence upon plant form. In the green-violet portion of the spectrum, where photosynthesis is least pronounced, plants grow as usual, while in the red-orange portion, where photosynthesis is most active, they become etiolated. Furthermore, Godlewski[3] obtained normal plants in the presence of light but in

[1] **Vöchting, Hermann,** Ueber die Bedeutung des Lichtes für die Gestaltung blattförmiger Cacteen. Zur Theorie der Blattstellungen. Jahrb. wiss. Bot. **26**: 438–494. 1894. [The same phenomenon is exhibited by some of the platyopuntias of southern Arizona.—*Ed.*]

[2] **Wiesner, J.,** Photometrische Untersuchungen auf pflanzenphysiologischem Gebiete. I Abt. Orientirende Versuche über den Einfluss der sogenannten chemischen Lichtintensität auf den Gestaltungsprocess der Pflanzenorgane. Sitsungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **102**^{I}:291–350. 1893.

[3] **Godlewski, Emil,** Zur Kenntniss der Ursachen der Formänderung etiolirter Pflanzen. Bot. Zeitg. **37**: 81–92, 97–107, 113–125, 137–141. 1879.

the absence of carbon dioxide. Thus, light of the shorter wave-lengths, and not the possibility of photosynthesis, is the requisite condition for normal form. This conclusion is also supported by the experiments of Vines,[1] who grew plants in light but in a soil without iron. Chlorotic plants were thus obtained, but their form was quite similar to that of normal plants, even though they were without chlorophyll and, consequently, could not assimilate carbon dioxide.

Only in certain plants is the shape of the leaves determined by the occurrence or non-occurrence of photosynthesis. Some etiolated leaves, such as those of wheat, contain little protein material and relatively large amounts of [dissolved or digestible] carbohydrates, while some other leaves, such as those of bean and lupine, are rich in protein material and contain almost no [dissolved or digestible] carbohydrates at all, excepting only a very little starch in the stomatal guard cells. The relative amounts of proteins in wheat and bean leaves, in the etiolated and normal condition, are given below, in percentage of total green weight. It thus appears that etiolated bean leaves

	Green	Etiolated
Wheat leaves	1.99	1.28
Bean leaves	4.95	8.38

contain more protein than do normal leaves, but they nevertheless remain small and undeveloped. As has been stated (page 228), the respiratory activity of etiolated bean leaves is very low, but respiration is greatly increased when sugar is supplied. Carbohydrates are necessary for the growth of all leaves, but in those of the bean and similar plants, where carbohydrates [aside from the celluloses of the cell walls] do not accumulate, these substances must, under normal conditions, be derived directly from photosynthesis. Thus bean leaves kept in darkness are deficient in carbohydrates and so cannot grow. Leaves of the other group of plants (such as wheat) are not dependent for their supply of carbohydrates at any particular time upon the rate of photosynthesis, for these substances accumulate in such leaves and the latter always contain much starch. Therefore wheat leaves, as has been seen, attain their usual size in darkness, or even become larger than in light.

The necessity of carbohydrates for normal leaf development is also shown by the experiments of Jost,[2] who obtained etiolated leaves of almost normal size in darkness, by supplying the needed nutrient materials. These etiolated leaves lived a long time in spite of the absence of light. When green leaves were placed in darkness, however, they degenerated rapidly, in spite of the fact that nutrient materials were supplied as in the other case. Jost suggests that perhaps the chlorophyll (or the whole photosynthetic apparatus) is subject to decomposition in darkness, thus giving rise to products that may be injurious to the cells in other ways.

Etiolated plants in darkness give off water at a lower rate than do green plants in light and, as has been mentioned, the decrease in transpiration rate brought about when plants are kept in a nearly water-saturated chamber exerts

[1] **Vines, Sydney Howard,** The influence of light upon the growth of leaves. Arbeit. Bot. Inst. Würzburg 2: 114–132. 1882.

[2] **Jost, Ludwig,** Ueber die Abhängigkeit des Laubblattes von seiner Assimilationsthätigkeit. Jahrb. wiss. Bot. 27: 403–480. 1895.

a marked influence upon form and structure even in light. It therefore appears that Palladin[1] is justified in supposing that etiolation in darkness is at least largely caused by diminished transpiration. The anatomical characters of etiolated plants are quite like those of plants grown in light but with water-saturated air, and all of the formal responses of etiolation appear to be explainable as resulting partly from alterations in the conditions controlling the rate of water loss and partly from the consequent alterations in the internal influences of the different organs upon one another. Thus, *Bellis perennis*, which forms a stem with spirally arranged leaves when grown in darkness, also shows the same response when grown in light with a water-saturated atmosphere.

Plants in which the leaves are slow to develop, such as the hop, form almost as long internodes in light as in darkness. In such cases stem growth is not influenced by leaf formation either in light or in darkness, so that the internodes can elongate freely.

Finally, Weber's[2] studies show that etiolated plants are poorer in ash, especially in calcium, than are green plants. Some results of Weber's analyses of etiolated and green pea leaves are given in the following table, which shows the total ash content and that of seven constituent elements (the latter reckoned as oxides), in percentage, on the basis of total dry weight in each case. In the same table are given similar results for bean leaves as obtained by Palladin.

Material Analyzed	Condition	Ash-constituents								Total Ash
		K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2	
Pea leaves	Green	4.85	0.11	3.21	1.02	0.09	1.67	1.64	....	12.77
	Etiolated	4.49	0.14	1.24	0.67	0.21	2.05	1.31	....	10.11
Bean leaves	Green	4.49	....	1.33	0.66	0.11	2.19	0.83	0.56	10.30
	Etiolated	3.42	....	0.26	0.40	0.03	3.25	0.12	0.06	7.54

Similar results were obtained by Schlösing from plants that had been grown with light but in a chamber with very moist air.

Among the conditions causing the structural peculiarities of etiolated plants are therefore to be considered: reduced rates of transpiration, the consequent modification in the distribution of water and dissolved mineral substances in the plant body, the non-occurrence of the photosynthetic process and (to some extent) light as such.

Some of the chemical reactions that are necessary for normal growth occur only in the presence of the blue-violet light rays. In the general influence of light upon plant growth and structure, many different kinds of reactions have

[1] **Palladin, W.,** Transpiration als Ursache der Formänderung etiolirter Pflansen. Ber. Deutsch Bot. Ges. **8**: 364–371. 1890. **Idem,** Ergrünen und Wachsthum der etiolirten Blätter. *Ibid.* **9**: 229–232. 1891. **Idem,** Eiweissgehalt der Grünen und etiolirten Blätter. *Ibid.* **9**: 194–198. **Idem,** Aschengehalt der etiolirten Blätter. *Ibid.* **10**: 179–183. 1892. **Idem,** 1893. [See note 1, p. 228.]

[2] **Weber, Rudolph,** Ueber den Einfluss farbigen Lichtes auf die Assimilation und die damit zusammenhängende Vermehrung der Aschenbestandtheile in Erbsen-Keimlingen. Landw. Versuchsst. **18**: 18–48. 1875.

been found to take part, such as oxidation, polymerization, decomposition, and even synthesis—the last in the presence of hydrocyanic acid, which is widely distributed in plants.[1] These processes are very rapid in the presence of inorganic salts.[2] They have not yet been studied in plants excepting in connection with the activity of chlorophyll, but there is no doubt that they must be important. Neuberg was right when he wrote: "These rapid chemical reactions caused by light may furnish a clue to the chemical processes that underlie phototropic responses, and even to the chemical nature of sunlight effects, in general upon both plants and animals" (Neuberg, cited just above).

It is well known that the seeds of certain plants germinate only in darkness,[3] while seeds of other plants, and certain spores, germinate only in light. In the latter case, as in growth phenomena generally, light acts not only as a stimulus that releases a reaction but also supplies energy that is necessary for the process in question. This statement seems to elucidate the fact, among others, that the light requirement of many seeds depends upon internal conditions, such as the stage of maturity of the seeds; light is especially requisite for the germination of seeds that have not been allowed to reach complete maturity. Many spores that ordinarily show a low percentage of germination in darkness germinate very well when iron salts of organic acids are supplied.[4] Finally, Wiesner's observations [see note 1, page 274] on the optimal light conditions (Lichtgenuss) for various plants have shown that the light requirement increases as the temperature of the surroundings falls. The various characteristic forms and structures resulting from etiolation are thus to be regarded as correlations between the different parts and organs of the plant, these being due partly to a deficiency in organic assimilation products, partly to a cessation of those photo-chemical processes that are independent of chlorophyll, and partly to a modified distribution, in the plant body, of water and dissolved mineral substances, which results from reduced transpiration. All these conditions must also influence the composition of the cell sap, which in turn controls turgor and the properties of the protoplasmic membranes.

Not only a complete lack but also an inadequate supply of light produces modifications in plant form and structure. If plants of the same species are grown, some in bright sunlight and some in diffuse light, the two groups exhibit very different structures, this difference being especially pronounced in the leaves.[5] Leaves grown in diffuse light are always thinner than those grown in

[1] **Ciamician, G.,** La chimica organica negli organismi. 99 p. Bologna, 1908. **Idem,** 1908. [See note 3, p. 34.]

[2] **Neuberg,** 1908. [See note 4, p. 34.]

[3] **Kinzel, Wilhelm,** Ueber den Einfluss des Lichtes auf die Keimung. ["Lichtharte" Samen. (Vorläufige Mitteilung.) Ber. Deutsch. Bot. Ges. **25**: 269–276. 1907. **Idem,** Die Wirkung des Liehtes auf die Kiemung. (Vorläufige Mitteilung.) *Ibid.*, **26**: 105–115. 1908. **Idem,** Lichtkeimung. Einige bestätigende und ergänzende Bemerkungen zu den vorläufigen Mitteilungen von 1907 und 1908. *Ibid.* **26**: 631–645. 1908. **Idem,** Lichtkeimung. Weitere bestätigende und ergänzende Bemerkungen zu den vorläufigen Mitteilungen von 1907 und 1908. *Ibid.* **26**: 654–665. 1908.

[4] **Laage, A.,** Bedingungen der Keimung von Farn- und Moossporen. Beih. Bot. Centralbl. **21**^{I}: 76–115. 1907.

[5] **Dufour, Leon,** Influence de la lumière sur la forme et la structure des fueilles. Ann. sci. nat. Bot. VII, **5**: 311–413. 1887.

direct sunlight, the palisade parenchyma being weakly or not at all developed in the former, while it is strongly developed in the latter (Fig. 135). Sunshine leaves have smaller epidermal cells with smooth lateral walls, while shade leaves have larger epidermal cells with wrinkled or wavy walls. These differences in the epidermal cells, between leaves grown in sunshine and those grown in shade, are so great that the two kinds of leaves might easily be regarded as belonging to entirely different species (Fig. 136).

In some cases very differently shaped leaves may be produced on the same individual plant by allowing some leaves to develop in sunshine and others in shade. *Campanula rotundifolia* may serve to illustrate this (Fig. 137). This plant usually produces two kinds of leaves: those near the base (which develop in spring, in the shade of surrounding plants) are rounded, kidney-shaped and borne on long petioles, while those on the upper part of the stem (which develop later, in strong light) are linear, pointed at base and apex, and without long petioles. If a plant bearing both sorts of leaves is kept for a time in very weak light the lateral buds on the *upper* part of the stem develop reniform, long-petioled leaves, like those normally occurring exclusively near the ground.

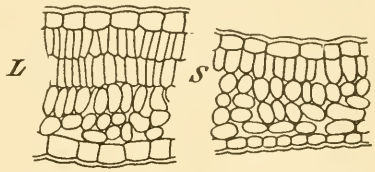


FIG. 135.—Cross-sections through leaves of *Fragaria vesca*, grown in direct sunlight (*L*), and in shade (*S*). (*After Dufour.*)

Although light is necessary for the normal development of green plants, they do not necessarily develop normally with continuous illumination; an alteration of periods of light and darkness seems necessary to produce structures such as occur in nature. Continuous illumination was obtained in the experiments of

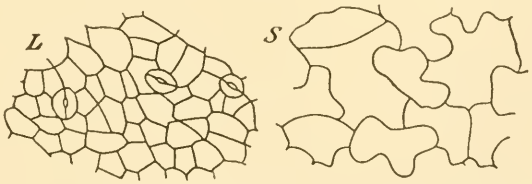


FIG. 136.—Surface view of upper leaf epidermis of *Tussilago farfara*, grown in direct sunlight (*L*), and in shade (*S*). (*After Dufour.*)

Bonnier[1] by means of electric arcs, the plants receiving no light but electric light through the entire six or seven months of their development. Some of these plants were lighted continuously, day and night, and others were darkened by means of opaque covers, for a period each day from 6 p.m. to 6 a.m. The injurious effect of ultra-violet light (which is relatively more intense in the light of the electric arc than in sunlight) was avoided by the use of clear glass screens, which of course absorbed the ultra-violet rays.

In these experiments, the plants that were darkened at night developed in the normal way and possessed normally differentiated tissues, but the con-

[1] **Bonnier, Gaston,** Influence de la lumière électrique continue sur la forme et la structure des plantes. Rev. gén. bot. 7: 241–257, 289–306, 332–342, 409–419. 1895.

tinuously illuminated plants, although they contained more chlorophyll, possessed a much simpler anatomical structure than the others, and resembled in certain respects, plants grown in continuous darkness. The leaves of *Helleborus niger*, for example, had normal structures when the plants were darkened every night; the mesophyll comprised the usual layer of palisade parenchyma above (containing most of the chloroplasts) with loose, spongy parenchyma below, the latter having numerous large air passages (Fig. 138, *J*). On the other hand, the Helleborus leaves grown with continuous illumination were very different from the others in several respects. Chloroplasts were here much more numerous than in the other case and they occurred almost throughout the entire tissue, instead of being mainly confined to the palisade. Instead of the loose, spongy parenchyma there was a tissue more like the fundamental parenchyma of growing regions, with almost no intercellular spaces at all (Fig. 138, *F*).

FIG. 137.—Upper portion of plant of *Campanula rotundifolia*, with reniform leaves developed from a lateral bud in diffuse light. (*After Goebel.*)

While photosynthesis is mainly dependent on the less refrangible half of the spectrum, normal growth and development require the more refrangible half (Fig. 132, curve *XY*). These more refrangible rays (blue and violet light) are strongly absorbed by plants. If, on a bright spring day, for example, the intensity of the blue-violet light is 666 in the open, it is only 21 in the shade of a fir tree, all but about one thirty-second of the energy of these rays having been reflected or absorbed by the leaves of the tree. Many formal characteristics of plants depend upon the intensity of the blue-violet light that reaches them. In evergreen plants, only the peripheral leaf-buds develop, since the interior buds are shaded, but in deciduous trees leaf-buds develop throughout the crown; in the latter case the tree is leafless at the time the buds are opening and all buds are at first equally lighted.[1]

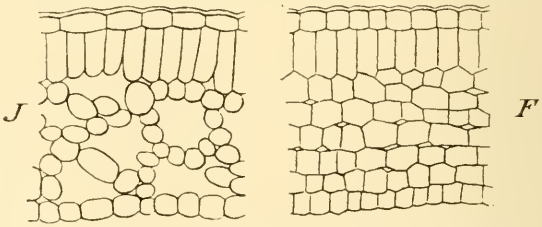


FIG. 138.—Cross-sections of leaves of *Helleborus niger*, grown in continuous light (*F*) and darkened during the night hours (*J*). (*After Bonnier.*)

Plants differ with respect to their light requirements and they may be

[1] Wiesner, 1893. [See note 2, p. 283.]

classified by this criterion, into shade plants and non-shade plants. In this connection the work of Wiesner has brought his term *Lichtgenuss* of plants into considerable prominence.[i]

By relative *Lichtgenuss*, Wiesner means the light income of the plant in question, expressed as a fractional part of the total sunlight intensity that might reach it if it were not shaded at all in its habitat. It is clear that the light income of different parts of the same individual, and of different individuals in the same natural habitat, is not a constant, but ranges between certain limits; either the maximum or minimum requirement of light intensity may be of interest (the limits of the range of light incomes under which a given species may thrive), or the average light income of an individual or group may be studied. The range of light intensities that a plant can bear, with which the work of Wiesner was most concerned, is a quantitative expression of the degree of the plant's adaptation for growing under various light conditions; it tells something of the internal conditions or properties of the plant as far as its light requirement is concerned. If the relative light genuss (relative photolepsy) of a plant is said to be 0.25, it is to be understood that that particular plant is growing in a shaded place where its light income is approximately 0.25 of what that income would be if all the shade were removed. Wiesner also employed what he terms the absolute genuss (absolute photolepsy, absolute light income, etc.), which is expressed in photometric units; he used the Bunsen-Roscoe unit.[1]

The ranges of the relative light incomes of several plants growing in their natural habitats at Vienna are shown below:

Buxus sempervirens (box)	1–1/100	(0.010)
Fagus sylvatica (beech)	1–1/80	(0.013)
Betula verrucosa (birch)	1–1/9	(0.111)
Larix decidua (larch)	1–1/5	(0.200)

[1] Wiesner, 1909. [See note 1, p. 274.]

[i] Although Wiesner expresses the hope that the term *Lichtgenuss* may eventually come to be an international technical word, it seems hardly probable that this hope will be realized. As an alternative he has suggested *photolepsy*. [Wiesner, J., Sur l'adaptation de la plante à l'intensité de la lumière. Compt. rend Paris 138: 1346–1349. 1904. Idem, 1907, p. 5 (see note 1, p. 274).] Whatever may be the pros and cons with reference to these two words, it is clear that neither one of them can ever have quite the same suggestiveness that *Lichtgenuss* has in German. In that language the word itself is familiar to every one and the technical meaning given it by Wiesner is derived from the ordinary meaning. To the non-technical German, *Lichtgenuss* carries a meaning very similar to that employed by Wiesner, while neither *Lichtgenuss* nor *Photolepsy* has any meaning at all to such a reader in most other languages. It therefore seems desirable to employ a simple and straightforward English word or phrase for non-technical purposes. *Light income* and *optimal light intensity* may be used. Neither of these has as much teleological implication as has the word *Lichtgenuss* in German. *Light income* means simply the amount of light actually impinging upon the plant in question. The *optimal light intensity* denotes the amount of light that must impinge upon the plant in order that it grow best, or most rapidly, etc. The *light requirement* of a given species is the range of light intensity within which that species can thrive, etc., being limited by a maximum and a minimum requirement. Measurements of light intensity should be recorded in absolute terms, not as percentages of the intensity of unobstructed sunshine at the given time and place, which itself varies greatly and rapidly at any locality and is often not at all the same for different places at the same time. Of the light actually reaching the plant surface only a part is absorbed, of course; much is directly reflected at the periphery and some usually passes through or is reflected from internal surfaces.—*Ed.*

These numbers may be regarded as the maxima and minima of relative light requirement for these plants. The relative minimum increases with the geographical latitude. *Acer platanoides*, for example, has a relative minimum light requirement of $\frac{1}{55}$ (0.018) at Vienna, $\frac{1}{28}$ (0.036) at Hamar, Norway, and $\frac{1}{5}$ (0.200) at Tromsö, Norway. Of course the light intensity in the open decreases with latitude, which suggests an explanation of this decrease in the relative minimum light requirement. Also, with lower temperatures the minimum light requirement is higher.

There is also a relation between the light income of plants and mycorhiza, the development of which occurs only in connection with plants that are confined to shady situations. Finally, the amount of chlorophyll in plants and the color of their leaves is related to the light income.

In one of his later papers Wiesner[1] expresses the two following conclusions: (1) Plants that are especially well adapted for growing in diffuse light are characterized by having their green parts (especially their leaves, which are strongly absorptive of light) so arranged as to receive light very freely; in many cases, indeed, the leaves are so placed as to receive the maximum intensity of diffuse light that the habitat affords. (2) Plants that are especially well adapted for growing in direct sunshine, on the other hand, are characterized by leaves and other green parts so placed as not to receive the light at its highest intensities, but to receive only the lower intensities.[k]

[1] **Wiesner, J.**, Ueber die Anpassung der Pflanze an das diffuse Tages- und das directe Sonnenlicht. Ann. Jard. Bot. Buitenzorg. Supplement 3^{I}: 48–60. 1910.

[k] In connection with the interpretation of all this work of Wiesner's it must be borne in mind that his measurements were made in terms of the effect produced, by the radiation studied, upon *photographic paper*. This paper is especially sensitive to light radiation of the shorter wave-lengths (blue-violet and ultra-violet), it is less sensitive to the medium wave-lengths of light (green, yellow) and is almost wholly unaffected by the long wave-lengths of light radiation (orange, red and infra-red). It is thus seen that the Weisner method automatically applies a relative weighting to the effect produced by each one of the different wave-lengths that constitute the radiant energy impinging upon the instrument, and the value obtained from any test is the integration of these weighted partial values. The relative sensitivities of the paper used might be experimentally determined for a variety of different short ranges of wave-lengths, and weighting coefficients might thus be determined for each range, by the use of which it might be possible to calculate from any given reading an approximate relative value for the actual radiation intensity as a whole, *providing all the tests dealt with radiation made up of intensities of the various wave-lengths in a constant set of proportions.* But the radiation to be studied varies from place to place and from time to time, not only in total energy content, but also in the relative proportions of the intensities of the various component wave-lengths; that is, in quality. From these considerations it becomes evident that the Wiesner method, for measuring and comparing the amounts of radiation received by plants in different places and at different times, must be regarded as crude and unsatisfactory, at its very best.

Besides this very serious physical objection to the method employing photographic paper, there must be considered another objection that is just as serious, based on physiological relations. For the purposes of ecology and physiology it is necessary, not only that the quality and intensity of the radiation received by plants in different places, etc., be measured and compared *as such*, but that the physical values obtained by such measurement be subjected to a physiological weighting, so as to give an index of the radiation received in each of the different habitats as it may affect plants growing therein. It is unnecessary to add that the sensitive-

There are many plants whose flowers open normally in darkness, so long as the rest of the plant is exposed to light. In some cases the form of the flowers produced is dependent upon light conditions. Thus, Vöchting[1] found that the formation of cleistogamous flowers (which are self-pollinated and never open) is markedly influenced by external conditions, especially by light. The plants of Vöchting's experiment were placed on the inner side of, and at various distances from, a northeast window, so that they received light of various intensities. With some plants the effect of being placed farther from the source of light produced only a decrease in the number and size of the flowers, but the flowers opened in all cases. In the case of plants with a tendency toward cleistogamy, however, the number of cleistogamous flowers produced increased as the plants were farther from the window. With such plants it is possible to obtain either ordinary or cleistogamous flowers at will, by controlling the light intensity during the flowering period.

The flowers of many plants are open only by day and are closed at night[2] (see Fig. 130, p. 279), while those of some other plants are open only at night and are closed by day. These periodic movements of petals and sepals are frequently dependent upon light variation, and, as is shown by measurements, they are directly due to unequal growth on the two sides of the organ. When growth of the outer or lower regions of the petals is more rapid, the flower closes, and opening occurs when growth is more rapid on the inner or upper side. Such movements of floral parts may also be brought about by temperature changes, to which many flowers are especially sensitive in this way; thus, a temperature change of 5°C. is sufficient to produce complete closing or opening of Crocus flowers.

Light also exerts an influence upon the development of lower plants, such as fungi.[3] Pilobolus, for example, develops normally in weak light but produces very long sporangiophores in darkness, where, also, the spores fail to mature. Light is injurious to colorless bacteria, which are killed by direct sunlight and hindered in their growth by diffuse light. This is shown very beautifully by H. Buchner's experiment. He pasted black paper letters on the bottom of a Petri dish containing a freshly prepared plate culture of typhus bacteria in nutrient agar, and then exposed the dish, bottom upward, to direct sunshine for one and a half hours. The dish was then placed in darkness for twenty-four hours, after which, when the black paper was removed, the forms of the letters could be plainly seen in the agar plate, because of the numerous white colonies that had developed, exclusively where the bacteria had been protected from the

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ness of photographic paper does not vary in the same way, with the wave-length of impinging radiation, as does the effectiveness of the radiation to favor plant growth and development. The problem is an exceedingly complex one, for which none but very general methods may even be suggested at present, but progress may be best furthered by a frank appreciation of the logical requirements.—*Ed.*

[1] **Vöchting, Hermann,** Ueber den Einfluss des Lichtes auf die Gestaltung und Anlage der Blüthen. Jahrb. wiss. Bot. 25: 149–208. 1893.

[2] **Pfeffer, W.,** Physiologische Untersuchungen. Leipzig, 1873.

[3] **Brefeld, [O.],** Ueber die Bedeutung des Lichtes für die Entwickelung der Pilze. Bot. Zeitg. 35: 386, 401–408. 1877.

sunlight by the black letters. The portions of the plate not thus protected were entirely free from living bacteria.

When bacteria are exposed to sunlight the majority of them are killed in the first few minutes of exposure. This was shown with twelve similar plate cultures of *Bacillus anthracis*, one of which was kept in darkness throughout the experiment, the other eleven being first exposed to sunlight for ten, twenty, thirty, etc., minutes, respectively, and then exposed to darkness for the rest of the culture period. When sufficient time had elapsed for the colonies to develop, these were counted in each of the plates. The following table shows the results of these counts.

Period of Exposure to Sunlight	Number of Colonies Developed	Period of Exposure to Sunlight	Number of Colonies Developed
minutes		*minutes*	
Not illuminated	2520	40	3
10	360	50	4
20	130	60	5
30	4	70	0

It is thus evident that light possesses very great disinfecting power,[1] and the Italian proverb, "Where sunshine enters not, there enters the physician," has a foundation in bacteriological science. Light is a potent factor in the automatic purification of polluted rivers. As they issue from cities, streams contain innumerable bacteria of many kinds, but before they have flowed far their waters become practically free from these organisms, through the action of sunlight. Water containing a hundred thousand cells of *Bacterium coli commune* per cubic centimeter was found to be entirely free of living bacteria after exposure to sunshine for a single hour. The ultra-violet rays (*rayons abiotiques*, of Dastre[2]) are especially injurious to colorless bacteria.

The colored bacteria are not affected by light as are the colorless ones. The purple bacteria studied by Engelmann are attracted toward brightly lighted portions of the medium in which they are growing, and they develop best in the presence of bright light.

§6. Influence of Gravitation on Growth and Configuration.[3]—That stems

[1] E. W. Schmidt has attempted to utilize the *sensitizing* action of fluorescent substances upon microörganisms, enzymes, etc., as a means of disinfection. In this connection see: **Tappeiner, Hermann, von,** and **Jodlbauer, A.,** Die sensibilisierende Wirkung fluorescierender Substanzen. Leipzig. 1907. **Schmidt, Ernst W.,** Enzymologische Mitteilungen. Zeitschr. physiol. Chem. **67**: 314–323. 1910.

[2] **Cernovodeanu, P.,** and **Henri, Victor,** Étude de l'action des rayons ultraviolets sur les microbes. Compt. rend. Paris **150**: 52–54. 1910. **Idem,** Comparison des actions photochemiques et abiotiques des rayons ultraviolets. *Ibid.* **150**: 549–551. 1910. **Urbain, Ed., Scal, Cl.,** and **Feige, A.,** Sur la stérilization de l'eau par l'ultraviolet. *Ibid.* **150**: 548–549. 1910.

[3] **Wiesner,** 1881. P. 85–130. [See note 2, p. 275.] **Idem,** Untersuchungen über die Wachsthumsbewegungen der Wurzeln. (Darwin'sche und geotropische Wurzelkrümmung.) Sitzungber. (math-naturw. Kl.) K. Akad. Wiss. Wien. **89**^{I}: 223–302. 1884. **Fitting, Hans,** Untersuchungen über den geotropischen Reizvorgang. Teil. I. Die geotropische Empfindlichkeit der Pflanzen. Jahrb. wiss. Bot. **41**: 221–330. 1905. **Idem,** same title. Teil II. Weitere Erfolge mit der intermittierenden Reizung. *Ibid.* **41**: 331–396. 1905. **Bach, H.,** Ueber die Abhängigkeit der geotropischen Präsentations- und Reaktionszeit von verschiedenen Aussenbedingungen. *Ibid.* **44**: 57–123. 1907. **Nordhausen, M.,** Ueber Richtung und Wachstum der Seitenwurzeln unter dem Einfluss äusserer und innerer Faktoren. *Ibid.* **44**: 557–634. 1907.

grow upward and that roots grow downward are such obvious facts that they remained uninvestigated for a long time. The first author to give this difference serious attention was Dodart,[1] and much work has been published in this connection since his time, but no real insight into these phenomena has even yet been obtained.

If a growing plant is changed from the vertical to the horizontal position, the root-tip soon bends downward and the tip of the stem upward. Knight[2] showed that this bending of growing plant organs is due to the influence of the force of gravitation. Seeds were allowed to germinate while attached to a rapidly rotating wheel. The axes of the seedlings assumed positions in the radii of the rotating disk, all of the main roots directing their tips outward while the tips of the main stems were directed inward. Here the force of gravitation was not allowed to act continuously upon the seedlings in any particular direction (since the axis of the wheel was horizontal), and in place of this force as it usually acts on plants was substituted the centrifugal force generated by rotation. The primary roots, which usually elongate in the direction of the pull of gravitation, now grew in the direction of the centrifugal pull; that is, toward the *circumference* of rotation. The primary stems, which usually direct their tips away from the center of the earth, grew in the direction opposite to that of the centrifugal pull; that is, toward the *center* of rotation.

The phenomenon of bending in response to the force of gravitation is termed *geotropism*. When the organ bends so as to direct its tip toward the center of the earth its geotropism is said to be *positive*, and when the bending occurs in the opposite direction it is said to be *negative*. Primary stems are generally negatively geotropic and primary roots are generally positively so.

The geotropism of lateral branches of both shoots and roots is less pronounced; these organs generally do not assume the vertical position, but take an oblique direction, more or less nearly approaching the horizontal. [They are said to be *apogeotropic* or *plagiotropic*.]

For the removal of the one-sided geotropic stimulus in experiments, various forms of *clinostat* are used, as well as the centrifuge already mentioned. The Pfeffer clinostat (Fig. 139) consists essentially of a metal axis (*c*) rotated by a clock-movement (*a*) and bearing at its free end the objects of the experiment. The axis may be arranged so as to have any desired position, horizontal, vertical, etc., the clock being correspondingly tilted and fastened by the screw *n*.

If a cork disk (*l*, Fig. 139, *B*) bearing germinating seeds is attached to the horizontal axis of a clinostat, with its plane surfaces perpendicular to the axis, and slowly rotated, the seedlings do not bend, but continue to grow in whatever direction they may have had when attached. The force of gravitation is of course not prevented from acting upon the plants in such a case, but the direction of this force is continually varied, so that during each revolution the gravity

[1] [**Dodart, [.**], Sur l'affectation de la perpendiculaire remarquable dans toutes les tiges, dans plusieurs racines, et autant qu'il est possible dans toutes les branches des plantes. Hist. Acad. Roy. Sci. 1700 (2nd ed.) : 47–63. Paris, 1741.]

[2] [**Knight, Thomas Andrew,** On the direction of the radical and germen during the vegetation of seeds. Phil. trans. Roy. Soc. London 1805 (Part I) [96]: 99–108. 1806.]

pull is applied as much to one side of the plant as to any other. Thus, if a certain region of the rotated plant lies underneath for a short time, this region soon comes to lie above for the same period, so that gravitation acts successively in opposite directions upon each portion of the plant, and a tendency to bend toward one side is offset by an equal tendency to bend toward the opposite side. Thus no geotropic bending occurs in such an experiment. Geotropic bendings are due to unequal growth on the two sides of the bending organ, and they occur only in the growing regions of stems and roots; after the tissues have become mature and have ceased to grow these bendings are no longer possible. Also, the more rapidly an organ is growing the more quickly it bends in response to gravitation, and all conditions that retard growth also retard the geotropic response.

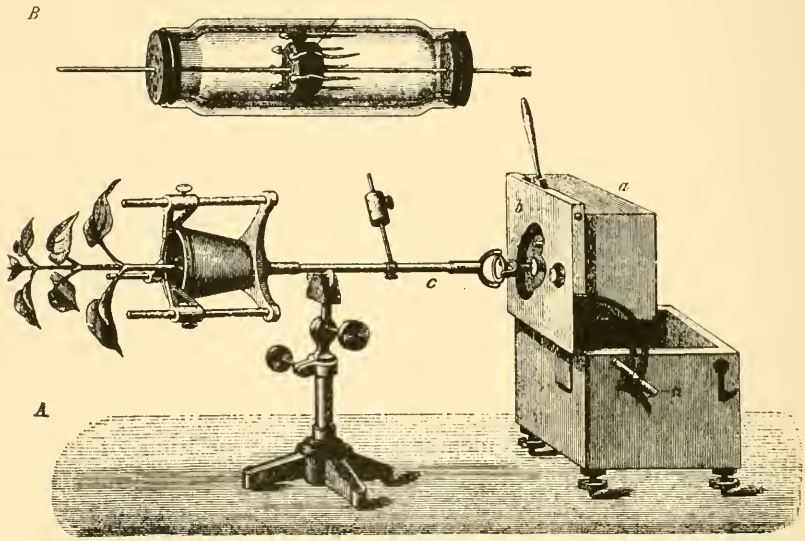

FIG. 139.—Pfeffer's clinostat. *A*, arranged for rotation of potted plant on horizontal axis; *B*, glass moist chamber, for rotating germinating seeds, etc.

The effect of gravitation upon the geotropically stimulated plant is to release certain chemical and physical reactions, and these, in their turn, lead to the bending itself, but only after a certain time has elapsed. The time period extending from the beginning of the application of the stimulus to the beginning of the visible response is termed the *reaction time*, and its length varies with different organs and plants, from about forty minutes to several hours. It is not necessary, however, that the stimulus be continued throughout all of this reaction period. If a plant is stimulated for a period shorter than its reaction time, as by lying quietly on its side, and is then rotated on the clinostat so as to equalize the lateral pull of gravity, geotropic bending finally occurs, providing the original period of stimulation was of adequate length. The shortest possible time of stimulation that is sufficient to bring about the later response is called the minimum *presentation time* of the geotropic stimulus. Generally this is only

from two to four minutes, rarely longer, and the fact that this period is so short is evidence in favor of the conclusion that the first effect of the stimulus is that of a release. By intermittent stimulation (by means of a specially constructed clinostat[1]) the presentation time may be made still shorter.

The angle assumed by leaves with reference to the stem is influenced by gravitation as well as by light. In Fig. 140, *B*, is shown a Coleus plant that has been rotated on a horizontal shaft parallel with its own axis for twenty-four hours. The leaves are seen to be bent backward toward the stem in a characteristic way. In the plant that has stood upright (Fig. 140, *A*) the leaves are nearly perpendicular to the stem.

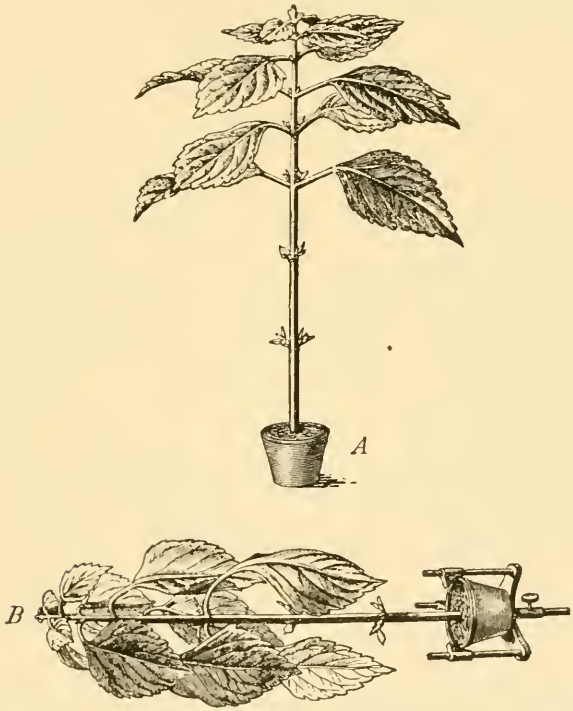

FIG. 140.—Coleus plants, in usual position (*A*) and after rotating for 24 hours (*B*), showing difference in leaf position. (*After Pfeffer.*)

Gravitation also frequently controls the position of floral parts,[2] as for instance the stamens and pistil of *Amaryllis formosissima*. When the flower bud opens under usual conditions these organs are directed downward (Fig. 141, at the left), but if the bud is allowed to open in an inverted position (Fig. 141, at the right), the stamens and pistil assume the same position with reference to the earth, but the *opposite* direction with reference to the remaining floral parts.

Plants that normally bear zygomorphic flowers may be made to produce actinomorphic ones if they are rotated in the proper manner during the development of the flowers. A zygomorphic flower is capable of being divided

[1] **Fitting**, 1905. [See note 3, p. 292.]

[2] **Vöchting, Hermann**, Ueber Zygomorphie und deren Ursachen. Jahrb. wiss. Bot. 17: 297–345. 1886.

into two symmetrical halves by but a single plane. Actinomorphic flowers, on the other hand, are symmetrical with reference to any plane passing through the floral axis, being really symmetrical about that axis. The flowers of *Epilobrium angustifolium* are zygomorphic when they develop normally (Fig. 142, at the left). If, however, a flowering shoot with young buds is slowly rotated about a horizontal axis, its own axis being parallel to that of the clinostat, then the flowers that open under these conditions are actinomorphic (Fig. 142, at the right).

The reasons why gravitation generally produces such different effects upon root and shoot, leading to positive geotropic bending in the one and to negative geotropic bending in the other, are to be sought in the organs themselves; these

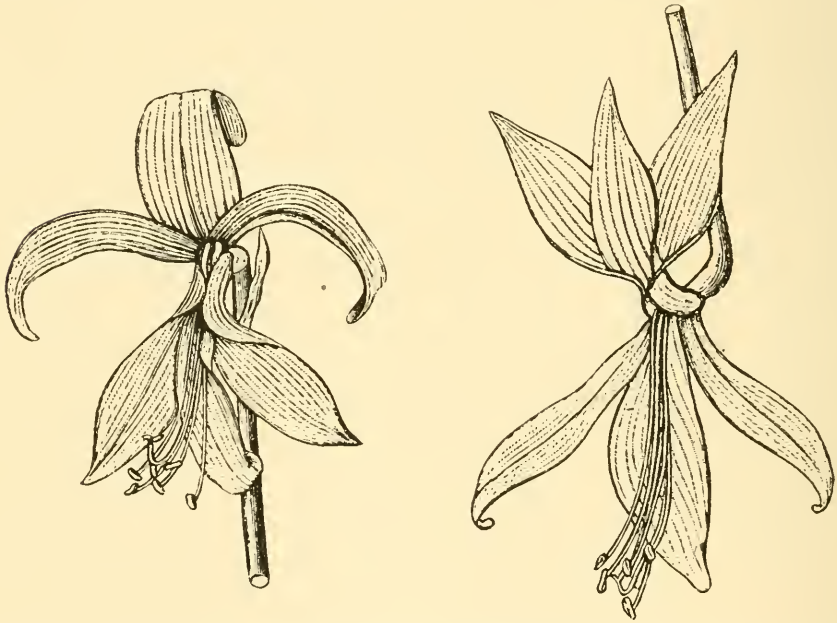

FIG. 141.—Flower of *Amaryllis formosissima* that has developed under normal conditions (at the left), and another that has developed from the bud in the inverted position (at the right); stamens and pistil are directed downward in both cases. (*After Vöchting.*)

organs are internally different and their various tissues are correlated in specific ways in each case. Similarly, the various responses of leaves grown in darkness are not due to the external light conditions alone, but must be related to special correlations between leaves and stem.

As has already been remarked, no insight into the fundamental nature of geotropic phenomena has yet been obtained. The suggestion of certain zoologists that the otocysts of lower animals serve, not as organs of hearing, but as organs of equilibration, has led some botanists[1] to seek such bodies in plants.

[1] **Haberlandt, G.**, Ueber die Perception des geotropischen Reizes. Ber. Deutsch. Bot. Ges. **18**: 261–272. 1900.

Němec[1] has advanced the idea that starch grains act as such "statoliths" in plant cells. The force of gravitation is thus supposed to act upon the starch grains, which are of higher specific gravity than the liquid about them, so that they always lie in that part of the cell nearest to the center of the earth (Fig. 143). The pressure exerted by these grains, upon the protoplasm of the cell, is supposed to inaugurate the series of protoplasmic changes which finally result in visible bending.

To this attempt at a physical interpretation, Czapek[2] has opposed a chemical one.[l] This writer was able to demonstrate certain chemical changes in tissues affected by geotropic as well as in those affected by phototropic stimuli. In this connection the observations of O. Richter[3] may be important, to the effect that negative geotropism disappears in plants under the influence of the more or less poisonous air of the laboratory (see also page 261).

Chemical investigation of growth phenomena is the only method of approach that promises to furnish a fundamental explanation of geotropic and

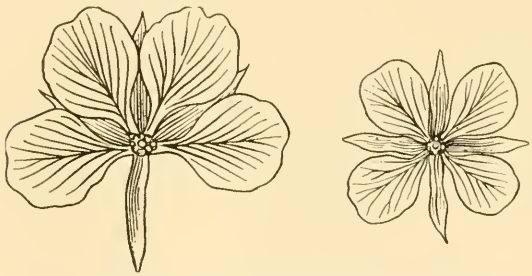

FIG. 142.—Normal flower of *Epilobrium angustifolium* (at the left), and actimorphic flower (at the right), the latter produced by rotation of the plant about a horizontal axis. (*After Vöchting.*)

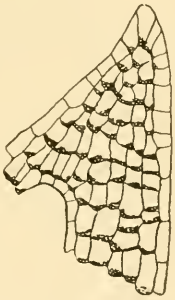

FIG. 143.—Tip of cotyledon of *Panicum mileaceum*, showing starch grains lying on the physically lower side of each cell. (*After Nemec.*)

phototropic reactions. For the present it can be said simply that under the influence of gravitation the primary shoot grows upward and the primary root downward.

[1] **Němec, B.,** Die Perception des Schwerkraftreizes bei den Pflanzen. Ber. Deutsch. Bot. Ges. 20: 339–354. 1902.

[2] **Czapek, F.,** Stoffwechselprocesse in der geotropisch gereizten Wurzelspitze und in phototropische sensiblen Organen. Ber. Deutsch. Bot. Ges. 20: 464–470. 1902. **Czapek, F.,** and **Rudolf, Bertel,** Oxydative Stoffwechselvorgänge bei pflanzlichen Reizreaktionen. (I. Abhandlung.) Jahrb. wiss. Bot. 43: 361–467. 1906. **Grafe, V.,** and **Linsbauer, K.,** Zur Kenntnis der Stoffwechselvorgänge bei geotropischer Reizung. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien. 119^{I}: 827–852. 1910.

[3] **Richter, Oswald,** Die horizontale Nutation. Sitzungsber (math.-naturw. Kl.) K. Akad. wiss. Wien 119^{I}: 1051–1084. 1910.

[l] To the editor there seems to be no opposition between these two views. The suggestions of Němec and Haberlandt attempt to explain only how the attraction of gravitation may become converted into a pressure of some cell-components upon others, and it is self-evident that this can represent only the first link in the chain of cause and effect that finally terminates in an alteration of growth rate in certain cells of the bending region of the plant. Between the pressure postulated by the physical theory and the bending itself, there must occur, as the author has already suggested, an unknown series of chemical and physical reactions, and Czapek's studies seem to deal with some of these.—*Ed.*

The following experiments show that gravitation acts only as a release, the conditions that control the phenomena of geotropic response residing in the plant itself. As has been mentioned, lateral roots do not exhibit positive geotropism, but are diageotropic, taking a position nearly horizontal when the axis of the plant is vertical. Bruck[1] has shown, however, that when the terminal 2 mm. of the primary root is cut away, thus putting an end to the elongation of this organ, then the laterals just above the wound become positively geotropic and bend vertically downward (Fig. 144). The same sort of response is observed in stems. In Fig 145 is shown the upper portion of a fir-tree (*Abies pectinata*) from which the tip has been broken away for some time. One of the lateral branches is seen to have become negatively geotropic and to have bent upward, just as if it were trying to replace the lost tip.

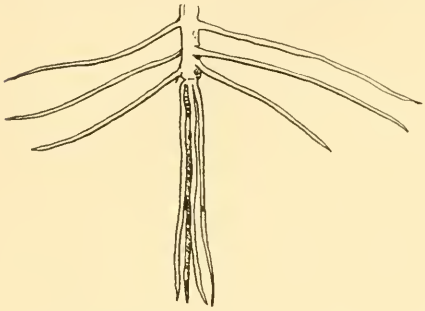

FIG. 144.—Root system from which the tip of the primary root has been cut away. The laterals nearest to the cut have become positively geotropic. (*After Bruck.*)

Errera[2] proposed to explain such phenomena as those just mentioned by postulating "internal secretions;" that is, special *hormones* that might regulate growth. In those cases where lateral roots or shoots take the place of primary ones, the apparent purposefulness of the response impresses itself upon some

FIG. 145.—Upper portion of tree of *Abies pectinata*. Removal of the tip of the main stem has made one of the branches negatively geotropic. (*After Errera.*)

minds so strongly that it is not easy for them to think of the chemical basis of the phenomena in question. There are other cases, however, where similar responses occur without the complication of what may seem like purposeful-

[1] Bruck, Werner, F. Untersuchungen über den Einfluss von Aussenbedingungen auf die Orientierung der Seitenwurzeln. Zeitsch. Physiol. 3: 486–518. 1904.

[2] Errera, L., Conflits, de préséance et excitations inhibitoires chez les végétaux. Bull. Soc. Roy. Bot. Belgique 42: 27–43. 1904–1905.

ness. Thus, Bässler[1] showed that, in plants that bear no lateral branches, decapitation of the main stem produces, within twenty-four hours, an upward bending of the leaves nearest the cut. The leaves may thus move through arcs of from 5 to 30 degrees, and even more in some plants. The reaction is more pronounced the nearer the leaves are to the wound. The wounding of the stem by a longitudinal incision fails to produce the response of leaf movement. Vöchting observed a still more remarkable case than those just mentioned. The removal of the inflorescence from a plant of *Brassica rapa* var. *oleifera* produced such a marked upward bending of the uppermost leaf that the latter became quite vertical.[2]

Plants can withstand a very rapid rotation upon the centrifuge, so that aleurone grains, starch grains and nuclei may be displaced in the cells; nucleoli may

FIG. 146.—Mycelium of *Mucor racemosus*, grown in sugar solution (*A*), and in peptone solution (*B*). (*After Klebs.*)

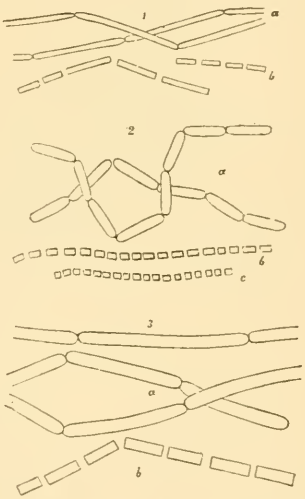

FIG. 147.—Diagrammatic representations of various forms of *Bacillus subtilis;* for description see text, preceding page. (*After H Buchner.*)

be thrown out of the nuclei and raphides may be made to penetrate through the cell walls.[3]

§7. Influence of Nutrition on Growth and Configuration.—If ordinary green plants are grown in mineral nutrient solutions, the nature and concentration of the solution determine not only the rate of growth, but also the configuration and the internal anatomy of the plant. This relation of developmental phenomena to the conditions of nutrition is still more clearly evident in the case of lower plant forms that are nourished by absorbed organic substances. *Mucor racemosus*, for example, produces thick hyphæ with blunt branches in sugar solu-

[1] **Bässler, Friedrich,** Ueber den Einfluss des Dekapitierens auf die Richtung der Blätter an orthotropen Sprossen. Bot. Zeitg. 67^{I}: 67–91. 1909.

[2] **Vöchting, Hermann,** Untersuchungen zur experimentellen Anatomie und Pathologie des Pflanzenkörpers. Tübingen. 1908. See Taf. 18, Fig. 2; Taf. 19, Fig. 9.

[3] **Andrews, Frank Marion,** Die Wirkung der Centrifugalkraft auf Pflanzen. Jahrb. wiss. Bot. 28:1–40. 1903.

tion (Fig. 146, *A*), but forms thin hyphæ with pointed branches in peptone solution (Fig. 146, *B*).[1]

The hay bacillus (*Bacillus subtilis*) shows pronounced polymorphism, according to the medium in which it grows.[2] In a slightly alkaline, 5-per cent. solution of beef-extract the cells are rod-shaped, 6–10 μ long and 0.5 μ in diameter (Fig. 147, 1, *a*). In neutral, 5-per cent. sugar solution, containing also 0.1 per cent. of beef-extract, the cells are shorter and thicker, 4–6 μ long and 0.8 μ in diameter (Fig. 147, 2, *a*). Very large cells are produced in hay infusion, 12 μ long and 1.0 μ in diameter (Fig. 147, 3, *a*). In all of these media cell division proceeds very rapidly, but the newly-formed cross-walls are so thin and so little refractive toward light that they cannot be seen at all excepting in stained preparations. When the rods above described are stained with iodine, each one is seen to be composed of a chain of much shorter cells (Fig. 147, 1, *b*; 2, *b*; 3, *b*).[3]

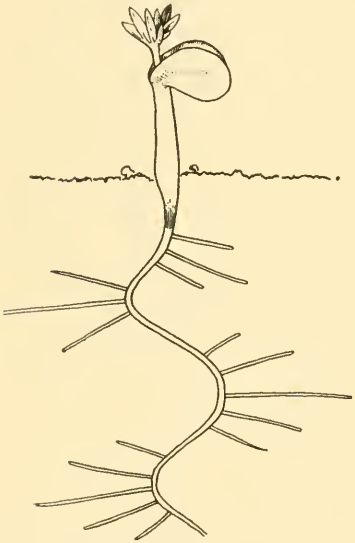

Fig. 148.—Lupine seedling with bent primary root, showing the formation of laterals exclusively on the convex side of each bend.

§8. Influence of Wounding, Traction and Pressure on Growth and Configuration.—Wounding of all sorts exerts a pronounced influence upon the rate of growth of plant organs; a wound may simply retard growth or may cause it to cease altogether. Wounding is frequently followed, also, by various kinds of bendings in growing organs. Especially noteworthy is the Darwinian response of roots, so called by Wiesner, in honor of Charles Darwin,[4] who first described this reaction. If a root-tip is laterally wounded (as by cutting, burning, etc.), the root bends, after a time, in the direction toward the uninjured side. Frequently this bending is so pronounced that the root-tip is carried upward and then downward again, thus forming a loop in the growing region. This response has sometimes been regarded as purposeful, since its *effect* is to remove the root-tip from a dangerous neighborhood. Wiesner[5] has shown, in later studies, that the Darwinian response is really a double one, being composed of two consecutive bendings in opposite directions. From twenty-five to forty-five minutes after the occurrence of the wounding a very slight bending takes place in the *upper* portion of the region of growth, the direction of this bending being such as to move the root-tip *toward* the position occupied, at the time of

[1] **Klebs, Georg,** Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.

[2] **Buchner, Hans,** Beiträge zur Morphologie der Spaltpilze. Nägeli's Untersuchungen über niederen. Pilze aus dem Pflanzen. München and Leipzig, 1882. P. 205–224.

[3] Also, compare the experiments of **Ritter,** 1907. [See note 1, p. 270.]

[4] [**Darwin and Darwin,** 1880. [See note 1, p. 314.]

[5] **Wiesner,** 1884. [See note 3, p. 292.]

wounding, by the object that caused the wound. This first response is so slight that it is to be demonstrated only by very precise observation. From forty-five to one hundred thirty-five minutes after the occurrence of the wounding, the second response begins, a bending in the *lower* portion of the growing region. This second bending of the root is in the direction opposite to that of the first, thus moving the root-tip as if to withdraw it from the wounding object. The second bending is more pronounced than the first and is of course the one studied by Darwin. The detailed mechanics of these bendings is still not understood.[m]

FIG. 149.—Witches' broom on leaf of *Pteris quadriaurita*, caused by the fungus *Taphrina laurentia*. (*After Goebel.*)

Under usual conditions the laterals are distributed evenly over the surface of the primary root, but when bends occur in the primary roots the secondary ones develop in each bent region only on the convex side (Fig. 148).[1]

Parasitic fungi often cause striking changes in plant form and structure. *Sempervivum hirtum* normally bears obovate leaves, about twice as long as broad. When infected with the fungus *Endophyllum sempervivi*, however, this plant produces leaves that are as much as seven times as long as broad. On various trees and shrubs frequently occur peculiar structures known as "witches'

[1] **Noll, F.,** Ueber den bestimmenden Einfluss von Wurzelkrümmungen auf Entstehung und Anordnung der Seitenwurzeln. Landw. Jahrb. **20**: 361–426. 1900.

[m] In connection with these traumatropic responses (or wound reactions), see: **Spalding, Volney M.,** The traumatic curvature of roots. Ann. bot. **8**: 423–451. 1894.—*Ed.*

brooms," strikingly modified branch systems, which are caused by parasitic fungi. A very interesting witches' broom is produced by the fungus *Taphrina laurentia* upon the fern *Pteris quadriaurita*, as is shown in Fig. 149. These curious outgrowths are always formed on the upper side of the leaf, and they grow upward in such manner as to suggest that another leafy plant has established itself upon the fern. They resemble similar lateral outgrowths found on the leaves of fossil ferns.

FIG. 150.—Flower-heads of *Crepis biennis;* two unmodified, and two modified by the presence of the mite Eriophyes.

From the point of view of plant phylogeny,[n] it is sometimes possible to throw light on genetic relationships by the study of pathological phenomena that may include the formation of atavistic structures. These latter may be apparently quite new for the plant in question, but may be actually like structures that were usual in its remote ancestors.[1] Thus, the compound flower-heads of *Crepis biennis*, when infected with the mite Eriophyes, are very different from the uninfected heads, and the modification appears to be an atavistic one, reverting to an ancestral type (see Fig. 150). Also, the diœcious plant *Melandryum album* bears perfect ("bisexual") flowers when infected with the parasitic fungus *Ustilago antherarum* (see Fig. 151).

As has been stated (page 251), some tissues in ordinary plants are subjected to traction, while others are subjected to pressure. An artificial pull may be applied to a plant, to determine the effect of traction upon growth. Hegler's[2]

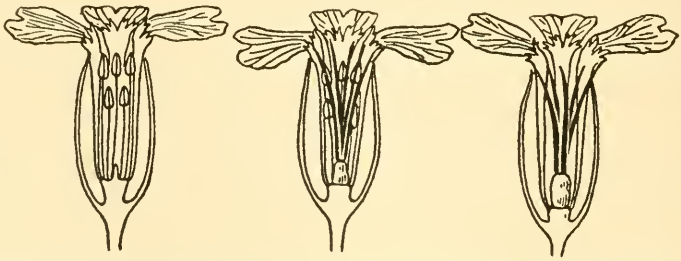

FIG. 151.—Flowers of *Melandryum album*, in vertical section. The normal staminate and pistillate flowers are shown at left and right, respectively, and the middle diagram represents a perfect flower (with both stamens and pistils), this modification being produced by the presence of the fungus *Ustilago antherarum*.

[1] **Potonie, H.,** Grundlinien der Pflanzen-Morphologie im Licht der Paleontologie. Jena, 1912.

[2] **Hegler, Robert,** Ueber den Einfluss des mechanischen Zugs auf das Wachsthum der Pflanze. Cohn's Beiträge zur Biol. d. Pflanzen. 6: 383–432. 1893. [**Newcombe, Frederick C.,** The regulatory formation of mechanical tissue. Bot. gaz. 20: 441–448. 1895. **Pieters, Adrian J.,** The influence of fruit-bearing on the development of mechanical tissue in some fruit-trees. Ann. bot. 10: 511–529. 1896.]

[n] This paragraph appears for the first time in the 7th Russian Edition.—*Ed.*

experiments may be mentioned as examples of this sort of study. A thread was attached to the tip of the shoot to be experimented upon, was passed over a pulley above, and bore a weight on its free end, the downward pull of the latter being transmitted so as to produce an upward pull upon the end of the shoot. The following table presents the results of some of Hegler's measurements of the daily rates of elongation of various plants with and without traction and with different amounts of traction.

Plant	Amount of Traction Applied	1st Day		2d Day		3d Day		4th Day	
		Rate of Elongation	Alteration in Rate Due to Traction	Rate of Elongation	Alteration in Rate, Due to Traction	Rate of Elongation	Alteration in Rate, Due to Traction	Rate of Elongation	Alteration in Rate, Due to Traction
	grams	*mm.*	*per cent.*	*mm.*	*per cent.*	*mm.*	*per cent.*	*mm.*	*per cent.*
Sunflower seedling	00	15.2	.....	10.7	.....	6.4	.....	3.5	.....
	50	8.2	−46.0	11.2	+ 4.7	6.9	+ 7.8	4.2	+20.0
Hemp seedling	00	10.2	.....	7.9	.....	5.6	.....	.....	.....
	20	4.0	−60.7	3.9	−50.6	6.1	+ 8.9	.....	.....
Dahlia shoot	00	21.1	.....	15.5	.....	9.3	.....	5.7	.....
	50	16.2	−23.2	17.1	+10.0	.....	.....	.....	.....
	100	.....	.....	.....	.....	7.9	−15.0	6.8	+19.3

The first effect of applying an upward pull to the plant is seen to be a pronounced retardation of growth, but the rate of elongation afterwards increases, if the same traction is continually applied, so as to equal and finally even to exceed the rate of the control plant without traction. Frequently, as with the sunflower seedlings and Dahlia shoots of the above table, the period of growth retardation lasts only about one day, but in some cases, as with the hemp seedlings, it lasts longer. If the traction is increased after the growth rate begins to surpass that of the control, a second period of retardation ensues, as is seen in the case of the Dahlia shoots,where the weight was increased at the beginning of the third day, from 50 g. to 100 g.

Traction is effective to modify the anatomical structure of plants as well as to produce alterations in the rate of enlargement.

Our knowledge of the effect of pressure upon plant growth has been much advanced by the work of Pfeffer,[1] who embedded growing plant parts in plaster of Paris or gelatine, and studied the pressures developed by growth, and their effects upon the tissues. According to the problem in hand, either the entire plant or just the growing region was embedded. Plaster of Paris proved very satisfactory in these experiments, since it furnishes a rigid material when it hardens and at the same time allows free access of both air and water to the

[1] Pfeffer, W., Druck- und Arbeitsleistung durch wachsende Pflanzen. Leipzig, 1893.

embedded organ. The pressures developed by growing plant tissues are considerable; the primary root of a bean seedling must be enclosed in a plaster cylinder from 1.0 to 1.5 cm. in diameter, if the bursting of the cast is to be avoided.

With the retardation of enlargement that occurs in organs confined in plaster casts, there occurs an acceleration in the development of the internal tissues and structural elements. In a bean root that has been thus embedded for from fifteen to twenty-seven days, fully developed spiral and pitted tracheæ are found at a distance above the root tip of only 1.6 mm., while in a similar root growing normally these vessels do not extend farther than to within from 25 to 35 mm. of the tip. In general, a transverse section from near the tip of such a confined root has the same appearance as a similar section taken from 30 to 50 mm. above the tip of a normal root.

When enlargement is not completely checked but is merely retarded, then the region of elongation is found to be shorter than in normally growing roots, in proportion to the growth-retardation to which the root has been subjected. The normal bean root has a region of elongation about 10 mm. long, while this region may frequently be only 5 or 6 mm., or even no more than 3 mm., long in roots in which growth has been artificially retarded by pressure.

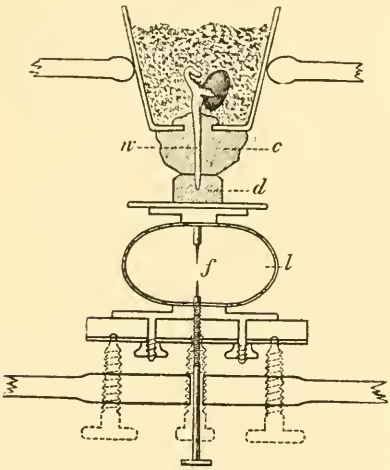

FIG. 152.—Pfeffer's apparatus for measuring the downward pressures developed by growing roots. (*After Pfeffer.*)

The experiments just described show that growing plant organs may develop relatively very great pressures as they react against obstacles to their growth. Pfeffer carried out a series of experiments to determine the magnitudes of the forces thus brought into play. Cubes of plastic clay were prepared, with small, shallow openings, into which the tips of growing roots were placed. The roots continued to elongate, in spite of the resistance offered by the clay, and penetrated into the cubes. Then iron replicas of the roots were forced into the same cubes into which the roots had penetrated, and the amount of pressure thus required was determined. From many tests Pfeffer concluded that this pressure was as great as from 100 to 140 g. for the Windsor bean. More precise measurements were made with a spring-dynamometer (Fig. 152). To keep the root from bending as the pressure developed, its upper portion was embedded in a fixed plaster of Paris block (Fig. 152, *c*). The tip was set in a movable plaster block (*d*) which was pressed downward as growth of the root occurred, thus transmitting the pressure of the growing organ to the spring (*l*) of the dynamometer. The following table presents the results of a number of experiments of this kind. In each case are given: the duration of the

experiment, the diameter and cross-sectional area of the root, the total pressure developed, and the pressure per square millimeter of cross-sectional area, the last both in terms of grams and in atmospheres. The total pressure divided by the cross-sectional area of the root is of course the pressure per square millimeter. The last value is then divided by 10.33 (the weight, in grams, of a mercury column 760 mm. high and with a cross-section of 1 sq. mm.), to give the pressure in atmospheres.

Experiment No.	Duration of Experiment	Root Diameter	Cross-sectional Area of Root	Total Pressure Developed	Pressure per sq. mm.	
	hours	*mm.*	*sq. mm.*	*grams*	*grams*	*atm.*
1	70	2.1	3.40	257.5	72.8	7.04
2	72	2.2	3.70	294.3	79.5	7.70
3	36	2.0	3.20	352.7	110.2	10.67
4	192	1.8	2.60	260.6	100.2	9.70
5	120	2.0	3.10	272.0	87.7	8.49
6	94	1.2	1.13	226.0	200.0	19.36
7	94	1.6	2.01	226.0	107.9	10.44
8	58	2.4	3.46	250.0	72.2	6.98
9	58	3.0	4.71	250.0	53.1	5.16

From these data it appears that the root of the Windsor bean (*Vicia faba*) may develop a downward pressure of from 226 to 352 g., or that it may exert a pressure of from 5 to 19 atmospheres.

Summary

1. **Influence of Temperature on Growth and Configuration.**—Other conditions being suitable for growth, each plant form grows most rapidly with a certain temperature (called its *optimum temperature* for growth). With lower or higher temperatures growth is less rapid, until the *minimum* or *maximum* temperature is reached, beyond which this process fails to occur at all. The minima, optima, and maxima differ for different plants. Considering plants in general, the minimum temperature for growth may be as low as 0°C., or even a little lower, and the maximum may be far above 50°C. (algæ of thermal springs). The optimum temperature for the growth of ordinary plants generally lies between 20° and 35°C. When plants are in an inactive condition (as in the case of dry seeds or spores), they can retain vitality through prolonged exposures to temperatures that are far above the maximum, or far below the minimum, for growth.

Within the limits of the range between the minimum and the maximum for growth, the *kind* of growth is greatly influenced by temperature, which is thus markedly effective in determining the configuration of the plant body. Temperature fluctuation is especially influential. One of the acetic acid bacteria forms short rods when grown with a temperature of 34°C., while filaments are produced in cultures grown with

a temperature of 40°C. Alpine forms of ordinary plants that grow also at lower altitudes are markedly different from the lowland forms, and this is partly due to the different temperature conditions of the two environments. The date of flowering is generally much later for plants grown in a cold region than it is for other individuals of the same form grown in a warm region, and this difference is related to differences between the two regions with respect to the temperatures of the preceding autumn and winter, as well as of the spring. Molisch introduced the warm-bath method for breaking the dormant period and forcing the early formation of flowers, in shrubs, etc. Shoots bearing dormant winter buds are submerged in warm water for half a day, after which treatment the buds immediately proceed to develop flowers under greenhouse conditions, while untreated shoots do not respond to the same conditions until the season is much farther advanced.

2. Influence of the Oxygen Content of the Surroundings on Growth and Configuration.—It appears that ordinary plants may be influenced, as to their rates of growth, by alterations in the oxygen content of the surrounding air. For microörganisms the oxygen supply is of the utmost importance. *Aerobes* (such as acetic acid bacteria) require oxygen for their development, while *anaerobes* can develop without it. *Obligate anaerobes* (such as butyric acid bacteria) are poisoned by oxygen, but *facultative anaerobes* (such as yeasts) thrive either with or without a supply of this element. Some aerobes require less oxygen than others. The mould Mucor develops the usual hyphal weft and sporangiophores when growing on the surface of a suitable medium, with plentiful air supply, but its growth is similar to that of yeasts when it grows at the bottom of a mass of liquid medium; this difference in configuration may be due to the difference in oxygen supply.

3. Influence of Other Gases on Growth and Configuration.—The carbon dioxide supply influences the growth of ordinary plants; it may be either too low or too high for healthy development. Some seedlings are very sensitive to small traces of ethylene and of other toxic gases in the air about them, and plants are generally injured by considerable amounts of illuminating gas in the soil surrounding their roots. If the supply is very low, some poisonous gases accelerate the growth of ordinary plants. Johannsen introduced the ether treatment for breaking the dormant period and forcing the early formation of flowers, in shrubs, etc. Shoots bearing dormant winter buds are enclosed for a number of hours in a chamber containing ether vapor, after which treatment the buds immediately proceed to develop leaves and flowers under greenhouse conditions, while untreated shoots do not respond to the same conditions until the season is much farther advanced.

4. Influence of Moisture on Growth and Configuration.—The rate of water supply to plant or organ must generally be somewhat greater than the rate of water loss, since growth is usually accompanied by the accumulation of water in the enlarging tissues. As already pointed out, the water supply for ordinary plants is from the soil, while transpiration represents the main form of water loss. Consequently, the moisture condition of a plant may be altered (1) by a change in the power of the soil to supply water to the roots, (2) by a change in the power of the roots to absorb water that is in contact with their external surfaces, (3) by a change in the power of the air (including the sunlight effect) to remove water vapor from the foliage, etc., (4) by a change in the ability of the aerially exposed surfaces to hinder the evaporation of water (*i. e.*, to retard transpiration), or (5) by any two or more of these changes operating at the same time. If the environmental conditions are such that the mean value of the ratio of intake to outgo is less than unity, then the plant is unable to develop under

those conditions. Although the internal conditions that influence the value of this moisture ratio (2 and 4, above) are capable of great internal adjustment, yet this adjustment is limited for each plant form; consequently some forms require a moist climate, others a dry one, etc. Plants that thrive in dry periods are characterized by structures that facilitate water absorption and hinder transpiration. Within the limits of its capacity for internal adjustment, the same plant form may develop under widely different moisture conditions. With relatively humid conditions, the internodes are generally long and the leaf blades are extensive, the cuticle is thin, and there is not much woody tissue, etc. With relatively arid conditions, the internodes are generally shorter and the leaves are smaller, the cuticle is thicker, and there is a greater development of woody tissue. Arid conditions often promote the formation of thorns and spines—also the development of succulence (as is usual for cacti). In some plants the morphological characters are so much influenced by the moisture conditions of the surroundings that two individuals, one grown in an arid and the other in a humid climate, often appear to be quite different species. Some plants that develop partly under water and then extend into the air produce very different structures in the two environments, and this difference seems to be largely related to moisture conditions.

As to the manner in which external moisture conditions influence plant growth and development, a few considerations may be mentioned. Growth by enlargment can not occur in cells that are not turgid, and the rate and kind of growth that occur depend upon the degree of turgidity present. The turgidity of every cell is inversely proportional (other conditions being constant) to the resistance offered to water absorption or to the forces tending to remove water from the cell. The relation of the rate of water supply to that of water loss determines whether turgidity shall increase, decrease, or be maintained.

If the environmental moisture conditions remain constant, on the other hand, the turgidity (and growth) of any cell may be altered by internal changes, such as alterations in the permeability of wall or protoplasm to water, or alterations in the osmotic and imbibitional attraction for water, exerted by the cell contents. Such internal changes are promoted by alterations in the amounts of various dissolved substances within the cell, such as acids, salts, etc.

The transpiration rate largely determines the rate of entrance and transport of dissolved materials from the soil (when the soil moisture supply is adequate and the cell membranes are permeable to these solutions); the chemical content of any tissue is therefore partially controlled by the water conditions of the surroundings, and the chemical content of a cell markedly influences its growth. The kinds and amounts of substances dissolved in the soil water exert potent influences on the growth of ordinary plants.

The roots of many plants are hydrotropic. When exposed to unlike moisture conditions on opposite sides, the root elongates more rapidly on the drier side, thus producing a bending of the root away from the drier environment. This response to one-sided moisture conditions is due to *hydrotropism*.

5. Influence of Light on Growth and Configuration.—Light conditions are very important in determining not only the rate but also the kind and extent of growth in ordinary plants. Stems generally elongate less rapidly by day than by night, and this seems to indicate that ordinary daylight retards stem elongation. Plant stems in continuous darkness elongate more rapidly and produce longer internodes than do those in light or with the natural day-night fluctuation, but leaves generally remain very small in maintained darkness and expand to their regular size only when light is

present at least part of the time, as in nature. Stem elongation appears to be differently retarded by different ranges of light wave-lengths; for wave-lengths about like those of sodium light (yellow) the retardation is least, notwithstanding the fact that the greatest intensity of sunlight generally occurs in this region of the spectrum. Retardation is greater with wave-lengths corresponding to red and green light, and it is greatest with wave-lengths corresponding to violet light.

Prolonged absence of light induces *etiolation* in ordinary plants. Etiolated plants have yellow leaves. If they produce stems, these are white or yellowish and have unusually long, slender internodes, with only rudimentary leaves. The potato sprout grown in darkness is an example of this kind of etiolation. Such plants as Bellis and Sempervivum, which form rosettes under ordinary light conditions, produce long stems in darkness, with spirally arranged, rudimentary leaves. The youngest internodes of twining plants, when subjected to natural light fluctuation, are usually in a condition that closely resembles etiolation; they are therefore not greatly different when grown in maintained darkness. Under ordinary conditions these shoots subsequently become green, and the leaves develop and become green in the usual way; they are thus "normally etiolated" only when young.

The etiolation of ordinary plants is prevented by an adequate supply of light that has wave-lengths corresponding to blue and violet light. But etiolation generally occurs if the plants are grown in red-yellow-orange light—that is, in sunlight from which the shorter wave-lengths have been removed or very much weakened. This relation of etiolation to light wave-lengths is not directly connected with carbohydrate photosynthesis. In some cases, however, the behavior of etiolated leaves seems to be related to the supply of soluble carbohydrates.

It appears that etiolation is brought about through the operation of several conditions, among which are to be mentioned: low transpiration rates and resultant modifications in the absorption and distribution of water and salts, low carbohydrate supply (especially to leaves), and absence of the direct effects of light, as such. It seems certain that many essential photochemical processes occur in illuminated plants, besides the one by which carbohydrates are formed, which alone has been much studied.

Plants subjected to the natural alternation of day and night develop very differently according to the intensity of the light they receive in the daytime, as well as according to the relative lengths of the day and night periods. Leaves that receive only diffuse light during the daytime are generally thinner, with less palisade tissue, than leaves of the same plant form receiving direct sunlight during their periods of illumination. Shade-grown plants exhibit other characteristic differences from sun-grown plants, and shade-grown leaves or branches are in many cases markedly different from sun-grown leaves or branches of the same individual. Plant forms differ with respect to their light requirements and with respect to the intensity of light they are able to bear; they may be classified roughly into shade plants and non-shade plants.

Lower forms of plants, such as fungi and bacteria, are influenced by light. Pilobolus grows healthily only when exposed to diffuse light during the daytime. The colorless bacteria (*e. g.*, the typhoid bacillus) are killed by a brief exposure to direct sunlight. Sunlight (especially the ultra-violet rays) is a potent influence in the purification of the water of rivers polluted by sewage.

Ability to respond to one-sided illumination, the response being more rapid enlargement on one side of the responding organ than on the other, is called *phototropism*, or *heliotropism*. With *positive phototropism* the organ bends toward the more *strongly*

illuminated side; with *negative phototropism* it bends toward the more *weakly* illuminated side. Positive phototropism is common in stems (including flower scapes) and frequent in leaves. It is pronounced in the sporangiophores of the fungus Pilobolus. Negative phototropism occurs in many tendrils and in some roots, especially aerial ones. In many leaves the phototropic response is such as to bend or twist the petiole so as to place the upper surface of the blade perpendicular to the axis of strongest illumination. Leaf mosaics arise from phototropic bendings. Some leaves (of "compass" plants) respond to strong, direct sunlight by bendings and twistings of the petioles or leaf bases in such manner that the surface of the leaf-blade comes to lie parallel to the direction of the direct solar rays.

Since phototropic bending results from unequal enlargement on the two opposite sides of the organ, the response occurs only in the region of enlargement, where the tissues are in the second phase of growth. The sensitive region, in which the one-sided illumination sets up the primary protoplasmic disturbance that leads to the response, is usually distinct and separated by a considerable distance from the bending region.

Phototropic bending, like the retardation of enlargement by light, is most markedly promoted by light of short wave-lengths (violet region of the sunlight spectrum). Wave lengths corresponding to yellow light are without phototropic effect, but the longest wave-lengths (red region of the spectrum) are somewhat effective.

6. Influence of Gravitation on Growth and Configuration.—Gravitation influences the form of plants especially through *geotropic* bendings. Like other bendings due to tropisms, these are produced by more rapid enlargement on one side of the bending organ. Not only roots and stems, but also leaves, flowers, and floral parts, are influenced in their positions and forms by gravitation.

Gravitation acts continuously on all objects, and the pull is always toward the center of the earth, but a plant organ may have any direction as related to the axis and direction of gravitation. Different organs that exhibit geotropism differ with respect to their equilibrium positions—that is, the positions in which they continue to grow equally on all sides in spite of the one-sided pull of gravitation. *Positively geotropic* organs (like primary roots) bend in such a way as to direct the tips *toward* the center of the earth, while *negatively geotropic* organs (like the primary shoots of many plants) bend so as to direct the tips *away from* the center of the earth. Primary branches of roots and shoots are generally in the equilibrium position with respect to gravitation when the tips point in a direction forming an angle with the axis of gravitation; these are said to be *apogeotropic* or *plagiotropic*. Root branches usually bend so as to direct the tips outward from the main root and *downward*, while shoot branches usually bend so as to direct the tips outward from the main shoot and *upward*. The *effect* of gravitation may be avoided with organs that are not in their equilibrium positions (and bending may thus be prevented) if the plant is slowly rotated upon a *clinostat* with horizontal axis. The clinostat may be rotated rapidly, so as to act as a centrifuge, in which case the effect of gravitation is removed and the effect of centrifugal force is made manifest. Knight's experiment demonstrates that centrifugal force acts like gravitation in its influence on plant bending. When thus treated, positively geotropic organs bend so as to direct the tips toward the *circumference* of rotation, *with* the centrifugal force, while negatively geotropic organs bend so as to direct the tips toward the *center* of rotation, *against* the centrifugal force.

In order that geotropic bending may occur, the organ in question must exhibit geotropism, its sensitive region (tip of root or stem) must be in a position other than that of its geotropic equilibrium, this region must remain in that position for a certain

time (*presentation time*), and the bending region (situated a considerable distance back from the tip) must be enlarging. After a period considerably longer than the presentation time (*reaction time*) bending occurs in the bending region. In clinostat experiments the primary protoplasmic disturbance of stimulation may be started by stationary exposure for a period somewhat longer than the presentation time, and then slow rotation may begin and continue. In such experiments bending occurs after the lapse of the reaction time; the period from the end of the presentation time to the end of the reaction time is called the *latent period.*

The hypothesis has been advanced that when the sensitive region first comes to be out of its equilibrium position some heavier cell components (*e. g.*, starch grains) begin to settle through the somewhat viscous cell contents, finally (at the end of the presentation time) coming to be on the physically lower side of each cell. This shifting of starch grains, etc., is supposed to release some form of process, which may be the first of a series or chain of chemical and physical disturbances, the final one of which acts directly on the cells of the distant enlarging (bending) region and produces unequal enlargement on the opposite sides. The latent period is here supposed to be the time necessary for the protoplasmic disturbance to be transmitted from the sensitive to the bending region. The differences in direction between the bendings produced by the same gravitational pull (between positive and negative geotropic bendings, etc.) must be due to internal differences in the bending organs themselves.

7. Influence of Nutrition on Growth and Configuration.—The nutrition supply, both of organic substances and of salts, exerts great influence on the rate and kind of growth. Especially in lower forms, such as moulds and bacteria, different nutrient media may produce pronounced morphological differences.

8. Influence of Wounding, Traction, and Pressure on Growth and Configuration.—Wounding of an enlarging tissue may retard or check enlargement, and therefore result in bending. The Darwinian (*traumatropic*) response of roots is a bending away from an object that wounds the tip, the bending itself occurring in the region of elongation above the tip. Some of the tissue strains in a bending primary root appear to favor the production of branches; the latter more often arise on the convex than on the concave side of a bend, or than on unbent portions of the primary root. Parasitic insects or fungi often cause striking structural peculiarities in the host plant (*e. g.*, "witches' brooms"). Some diœcious plants bear perfect flowers when infected with the right fungus. Traction (as by thread, pulley and weight) may greatly modify the rate and kind of growth in ordinary plants. External pressure that hinders or checks enlargement (as when a growing root is enclosed in a rigid plaster cast) has marked influence on the maturation of the tissues.

Pfeffer's experiments showed that the downward pressures exerted by enlarging roots may be very great, as great as 350 grams in a root of Windsor bean, or 200 grams (over 19 atmospheres) per square millimeter of the cross section of the root.

CHAPTER IV

TWINERS AND OTHER CLIMBING PLANTS

§1. **Twiners.**[1]—The stems of many plants are so slender and so weak mechanically that they cannot grow upright unless they climb upon supporting objects. Without such mechanical support these plants always creep upon the ground. Climbing plants grow up into the air by twining about, or attaching themselves to, other plants or any available support, and they are thus able to attain the best illumination.

Twiners have long, slender stems, the growing tips of which twine about suitable objects that happen to be near. Familiar examples of twiners are the hop (*Humulus lupulus*), the scarlet-runner bean (*Phaseolus multiflorus*), various species of Convolvulus (morning glory), and also some Polygonum species, as *P. dumetorum* and *P. convolvulus* (bind-weed). The terminal portion of a twining stem of *Humulus lupulus* is shown in Fig. 153. In all twining plants the growing tip moves about the axis of the older part, describing a more or less circular path; the direction of this movement is clockwise in some plants, and counter-clockwise in others (Fig. 154). In most plants the moving portion consists of the last two or three internodes. The time required for a complete revolution varies with the plant as well as with the environmental conditions. In one experiment this time period was found to be one hour and seventeen minutes for *Scyphanthus elegans*, one hour and forty-two minutes for *Convolvulus sepium*, one hour and fifty-seven minutes for *Phaseolus vulgaris*, and nine hours and forty-five minutes for *Lonicera brachypoda*. The circular movement of the terminal region continues until some solid object, such as the stem of another plant, is encountered and then the twiner begins to wind itself about the support, providing this is of suitable shape and size.

The turns of the resulting spiral are not closely applied to the support at first, especially if the support is very slender; later, however, the spiral elongates and becomes narrower, and the stem thus becomes firmly bound about the supporting object. A firmer hold is effected by the stiff hairs that are frequently present on the stems of twiners.

Twining plants are able to wind about very slender objects, but the diameter of the support must not be too great, or twining is prevented. The maximum diameter of the support varies with different plants; *Phaseolus multiflorus* twines about a support from 7 to 10 cm. in thickness, but twining fails to occur if the diameter of the support is as great as 23 cm. Many tropical twiners can twine about thick supports.

[1] **Darwin, Charles R.**, Movements and habits of climbing plants. 2nd ed., revised. London, 1875. **Baranetzki, J.**, Die kreisförmige Nutation und das Winden der Stengel. Mém. Acad. Imp. Sci. St.-Pétersbourg *VII*, 31 *VIII*: 1–73. 1883. **Pfeffer, W.**, Zur Kenntnis der Kontaktreize. Untersuch. Bot. Inst. Tubingen **1**: 483–535. 1881–1885. **Voss, Wilhelm**, Neue Versuche über das Winden des Pflanzenstengels. Bot. Zeitg. **60** *I*: 231–252. 1902. [**MacDougal, D. T.**, Practical text-book of plant physiology, XIV + 352 p. New York, 1901. **Pringsheim**, 1912. (See note 1, p. 253.)]

If a twining plant is placed upon a clinostat and slowly rotated about a horizontal axis, the twining movement ceases and growth proceeds in a direction parallel to the axis of rotation, while the younger turns of the previously-formed spiral become straightened out. Such experiments indicate that a geotropic response is necessary for twining.

§2. **Non-twining Climbers.**[1]—The long stems of non-twining climbers are

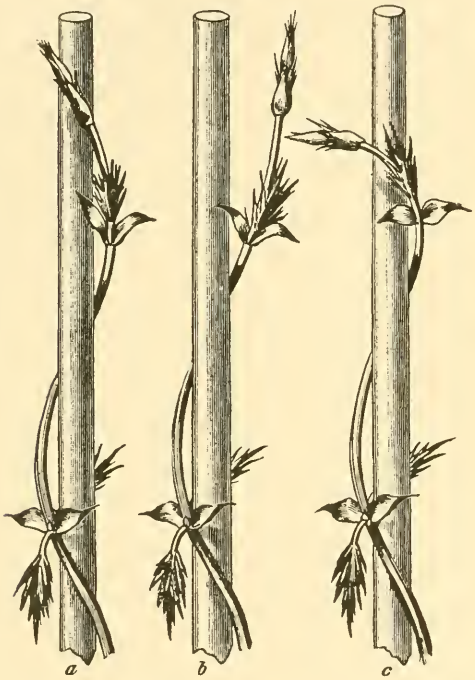

FIG. 153.—Twining stem of *Humulus lupulus*, in successive stages of movement. (*After Pfeffer.*)

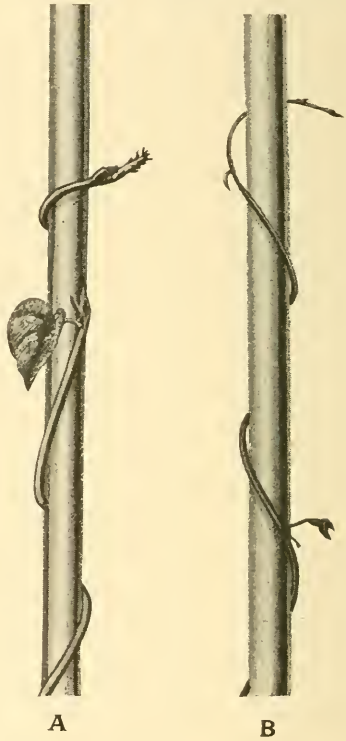

FIG. 154.—*A. Pharbitis*, shoot showing counter-clockwise twining. *B. Myrsiphyllum asparagoides*, shoot showing clockwise twining. (*After Bonnier.*)

unable to twine, but they climb by means of hairs, thorns, aerial roots, tendrils, etc. Tendrils are the most frequent of these special structures. These are morphologically different in different plants; in some forms (Vitis, Ampelopsis, the Cucurbitaceæ) they correspond to twigs, while in others they are leaves; thus the upper part of the pea leaf is a tendril while the pinnately arranged leaflets of the lower part are quite like those of ordinary leaves.

[1] **Darwin, Charles,** 1875. [See note 1, p. 311.] **Vries, Hugo de,** Längenwachsthum der Ober-und Unterseite sich krümmender Ranken. Arbeit. Bot. Inst. Würzburg, **1**: 302–316. 1874. **Schenck, Heinrich,** Beiträge zur Biologie und Anatomie der Lianen im besonderen der in Brasilien einheimischen Arten. 2 v. Jena, 1892–1893. [**Lengerkin, August von,** Die Bildung der Haftballen an den Ranken einiger Arten der Gattung Ampelopsis. Bot. Zeitg. **43**: 337–346, 353–361, 369–379, 385–393, 401–411. 1885. **MacDougal, D. T.,** Mechanism of the curvature of tendrils. Ann. bot. **10**: 373–402. 1896. **Idem,** 1901. (See note 1, p. 311.) **Pringsheim,** 1912. (See note 1, p. 253.)]

Young, actively growing tendrils nutate, so as to become hook-shaped. When the tendril comes into contact with a solid object coiling begins near the tip, so that it wraps itself about the support, if this is of suitable size and shape. This coiling movement results from unequal growth on the two opposite sides, brought about as a response to the stimulus of contact. The portion of the tendril that lies between the point of attachment and the base does not remain permanently straight but subsequently also becomes coiled, in the form of a spiral spring, so that the plant is drawn nearer to the support. The stem is thus not supported by a straight and inelastic suspension, which might be easily broken, but it hangs upon a coiled spring, which stretches a little when the plant is moved by the wind, thus largely avoiding the possibility of breaking. The tendril can become attached to a support only while still growing, and those that have not come into contact with a suitable support during the growing period generally wither and fall away. Fig. 155 represents a portion of the stem of *Bryonia dioica*, with a tendril attached to a twig of another plant. The middle portion of the tendril forms the spiral spring mentioned above.

FIG. 155.—Tendril of *Bryonia dioica*.

The tendrils of *Ampelopsis* behave in a peculiar manner when they happen to come into contact with an object about which they cannot twine, as in growing along a wall, for example. Some of the tendrils, which are appressed against the wall by a negative phototropic response and which continually nutate, happen to reach into crevices in the support. Such a tendril becomes thickened at the end within the crevice, so that it cannot be readily withdrawn, thus supporting the plant. [These tendrils also form adhering disks at their tips, by which they become attached to nearly smooth surfaces, (Fig. 156.)]

Investigations of the anatomy of tendrils show that these possess special arrangements that facilitate the reception of stimuli. The otherwise thick external walls of the epidermis of pumpkin (Cucurbita) tendrils, for example, are characterized by minute pits that extend the cell cavity outward, nearly to the outer surface of the wall, which is very thin at these points (Fig. 157, *A*). These pits are filled with protoplasm which is continuous with the protoplasm of the cell cavity itself, and these protoplasmic projections frequently contain small crystals of calcium oxalate, which have been thought to play a part in the propagation of the contact disturbance. These structures have been called *contact papillæ*. Such papillæ, of the external walls of the epidermis of a pump-

kin tendril, are shown in Fig. 157, *B*, where the shaded portion represents protoplasm. A crystal of calcium oxalate is shown embedded in the protoplasm of each papilla.

§3. **Circumnutation.**[1]—Darwin showed that all growing plants, although they seem to be elongating in a definite direction, are actually swinging about in more or less circular paths, but that these movements are so slow or so slight that they are usually quite unnoticed, without the employment of special methods of observation. Darwin thought that this sort of movement (which

FIG. 156.—Tendrils of *Ampelopsis heterifolia*. At the right a young tendril, with swollen tips; at the left, an old one with adhering disks, caused by contact with the wall, and coiled basal part. (*After Pringsheim.*)

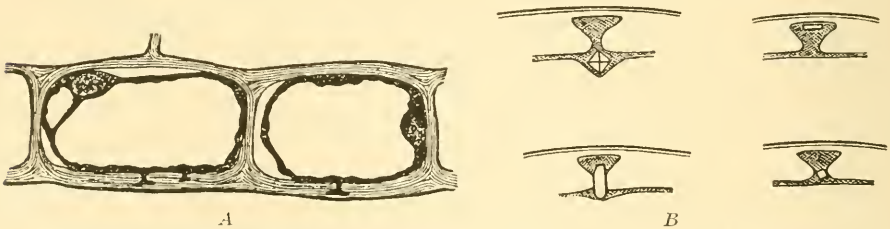

FIG. 157.—*A*, Epidermal cells from tendril of *Cucumis sativus*, showing protoplasm-filled pits in outer walls (contact papillæ). (*After Pfeffer.*) *B*, Contact papillæ of the outer walls of tendril epidermis of *Cucurbita pepo* (pumpkin). (*After Haberlandt.*)

he called *circumnutation*) is the fundamental, phylogenetically simple movement from which other plant movements due to unequal growth have evolved. Wiesner has maintained, however, that this hypothesis is not tenable; in many

[1] **Darwin, Charles R.,** and **Darwin, Francis,** Power of movement in plants. London, 1880. **Wiesner,** 1881. [See note 2, p. 275.]

growing organs, he failed to find any circumnutation.[a] Where it occurs it is caused merely by inequalities in the rate of elongation on different sides of the axis.

Summary

1. **Twiners.**—Climbing plants gain support by twining about objects or by forming mechanical attachments in other ways. Twiners have very slender stems, with long internodes. After the seedling stage of development is past, each new internode elongates very rapidly at first and the leaves of the youngest two or three nodes are retarded in their enlargement. Also, the youngest two or three internodes and the retarded leaf buds are pale in color. Consequently, this youngest region of the twining plant appears much as if it were etiolated, as has been said, although grown under natural day-night conditions of light. The rapidly elongating, younger portion enlarges in such a way that the tip is made to swing in a more or less circular path about the vertical axis, clockwise in some species and counter-clockwise in others. The time required for a revolution is, in general, between one and ten hours. If a suitable support is encountered (this must be nearly vertical and must not be of too great diameter), the elongating stem twines about it, each turn of the stem being higher than the preceding one. As elongation is about to cease, each internode straightens as much as possible, thus tightening the spiral on the support. At about this time the leaves expand, and the stem becomes green. Twining results from more rapid elongation on one side of the stem than on the opposite side, the lateral region of most rapid elongation migrating regularly around the stem. The regularity of this change is internally controlled, but twining ceases when the pull of gravitation is equalized about the plant axis by clinostat rotation.

2. **Non-twining Climbers.**—The stems of non-twining climbers behave much like those of twiners, except that they do not twine. They bear tendrils, aerial roots, etc., by which they become attached to any suitable support with which they happen to come into contact. In some forms tendrils correspond to leaves (pea), in other forms they correspond to branches (grape). Tendrils are sensitive to contact. They may grow around a suitable support by *thigmotropic* response, or they form adhering disks at their tips when stimulated by contact. After the tip is attached the main part of the tendril elongates very much throughout a narrow lateral region, thus producing a double spiral, which acts like a spiral wire spring. Many tendrils have contact papillæ, thin spots in the epidermal walls of the contact-sensitive region.

3. **Circumnutation.**—Charles Darwin showed that the tips of all vertically elongating stems swing slightly away from the vertical and around it, as elongation proceeds. He called this swinging *circumnutation.* To a very slight and frequently almost imperceptible degree, and in a very irregular manner, ordinary plant tips therefore act somewhat like the tips of twiners. Circumnutation is caused by an irregular and spasmodic migration, about the elongating portion of the shoot, of a lateral region of more rapid enlargement.

[a] Circumnutation is often very difficult to demonstrate, but it is doubtful if any shoot ever elongates with its own axis exactly vertical for more than momentary periods of time. It seems safe to regard circumnutation as universal in elongating stems. It is very irregular in many cases, and frequently very slight indeed.—*Ed.*

CHAPTER V

MOVEMENTS OF VARIATION[a]

§1. **General Survey of Plant Movements.**—All characteristic plant movements may be classified into two groups: the first includes *growth* movements, of growing organs, including *nutation* movements and *tropisms*, while the second embraces movements of mature organs, movements of *variation*. Only growth movements (the first group) have thus far been considered; as has been seen, they occur in growing organs and cease when growth is completed. Growth movements may, in their turn, be separated into two groups: in one are included movements that are produced by external conditions, such as movements due to phototropism and geotropism, which are called *paratonic* or *receptive* growth movements; in the other group are included *autonomic or spontaneous* growth movements, which are dependent upon the internal organization of the plant, such as the circular movements of twiners; and those due to epinasty, hyponasty, etc. Epinasty is the phenomenon of increased growth on the upper side of a leaf or stem, as compared to the lower side, and it results in the downward bending of the organ. Hyponasty denotes the opposite condition, where growth is more rapid on the under side of an organ. Both phenomena depend upon the internal organization of the organs in question, rather than upon specific stimuli from the surroundings. Movements of variation are also divided into paratonic and autonomic movements.

§2. **Autonomic Movements of Variation.**—Among the somewhat limited number of cases of autonomic movements of variation that are now known, the most striking example is found in the lateral leaflets of *Desmodium gyrans*,[1] the free ends of which move through an elliptical path. The rate of movement depends upon the temperature; with high summer temperature a complete circuit is completed in about three minutes. Similar, but much slower, movements may be observed in other plants; thus, the terminal leaflet of red clover (*Trifolium pratense*) completes its upward and downward movement in from one to four hours at summer temperatures.

§3. **Paratonic Movements of Variation.**[2]—The leaves of *Mimosa pudica*, (sensitive plant) which droop at a slight touch, furnish the best example of paratonic movements of variation. The leaf consists of a long petiole to which

[1] **Hofmeister, Wihelm F. B.,** Die Lehre von der Pflanzenzelle. Leipzig, 1867.

[2] **Brücke, Ernst,** Ueber die Bewegungen der Mimosa pudica. Müller's Arch. Anat. Physiol. u. wiss. Med. **1848**: 434–455. **1848. Pfeffer,** 1873. [See note 2, p. 291.] **Haberlandt, G.,** Reizleitendes Gewebesystem der Sinnpflanze. Leipzig, 1890. [**MacDougal, D. T.,** The mechanism of movement and transmission of impulses in Mimosa and other "sensitive" plants; a review with some additional experiments. Bot. gaz. **22**: 293–300. 1896.]

[a] In general, see: **MacDougal,** 1901. [See note 1, p. 311.] **Pringsheim,** 1912. [See note 1, p. 253].—*Ed.*

four pinnate leaflets are palmately attached; each of these leaflets consists, in turn, of a secondary petiole and rachis, which bears a large number of small leaflets of the third order (Fig. 158). The main petiole bears at its base a well-developed cushion or *pulvinus*, and organs of this kind occur also at the bases of the petioles of the leaflets of the second and third orders. A very slight touch upon the largest pulvinus is enought to cause the primary petiole to fall, and the leaflets of the third order to become erect with the upper surfaces of each pair of

FIG. 158.—Leaves of *Mimosa pudica*. *A*, normal position; *B*, after stimulation. (*After Pfeffer.*)

opposite leaflets against each other (Fig. 158, *B*). If the stimulus is strong enough it is propagated through the stem to the other leaves of the plant, both above and below, and these also fall and fold together. After a time the leaves gradually re-expand and regain their earlier positions. These phenomena occur in completely mature leaves, and they are entirely independent of growth.

Observations relating to details of the response here considered have shown that the movements of the leaf are caused by changes in the form of the pulvinus.

The main body of this organ[1] consists of parenchymatous tissue containing many intercellular spaces. The cell walls are markedly thinner in the lower half than in the upper half of the pulvinus. A vascular bundle transverses the central part. It can be shown that a very great tissue strain normally exists in the pulvinus, the exterior portion being under strong pressure while the inner part is stretched. This may be observed easily if the pulvinus or some part of it is cut out and placed in water, when the outer part expands, while the inner part contracts. The direct inference may be drawn from these facts, that the falling of the stimulated leaf is the result of a change in turgidity in the cells of the upper or lower half of the pulvinus.

If the lower half of the pulvinus is cut away as far as the vascular bundle, the petiole falls and remains in this position without again rising. If the upper half of the pulvinus is similarly removed, the petiole also falls subsequently, but it afterward erects itself and assumes a higher position than before. It therefore follows that the falling of the leaf is produced by a decrease in turgidity of the cells in the under half of the pulvinus, while the opposite movement is the result of a return of turgidity in these cells. That the leaf finally takes a higher position when the upper half of the pulvinus is removed is due to the fact that the cells of the lower half are now able to expand to a much greater degree than before the operation, since they encounter no resistance from the turgidity of the opposite portion. If a stimulus is applied to an inverted Mimosa plant, the leaves do not sink but begin to rise instead; that is, they move in the same direction, with reference to the stem and roots, as they did when the plant was upright. This rise is a result of the removal of resistance on the usually lower (now upper) side.

The decrease in turgidity of the cells of the normally lower half of the pulvinus is accompanied by a decrease in their circumference, a part of the water contained in these cells therefore migrates elsewhere. This water does not escape to the outside, for the surface of the cushion is dry after the response. It may be observed, however, that the pulvinus is dark-colored after the leaf falls, appearing as though injected with water. Brücke concluded that the water escaping from the cells passes into the intercellular spaces, displacing the air; when the stimulus is removed this water soon re-enters the cells and the intercellular spaces become refilled with air, thus rendering the pulvinus again light-colored.

The cause of the temporary extrusion of water by the cells of the lower half of the pulvinus is naturally to be attributed to changes in the properties of their protoplasmic membranes, brought about as a result of the stimulus. The exact nature of these changes is still unknown. The response of the Mimosa leaf, which is one of the indications that the plant is alive, occurs only under conditions that are favorable to the life-processes in general; there must be the proper kind and intensity of the temperature and moisture conditions, and oxygen must be supplied from the surrounding atmosphere. Chloroform anes-

[1] The pulvinus of the primary petiole is best for this kind of investigation and all the experiments here described have reference to this organ.

thetizes Mimosa and causes the plant to lose its power of reaction for some time.

The leaves of many other legumes, as well as those of some species of Oxalis, also respond to stimuli much as does Mimosa, but their sensitiveness is not nearly so pronounced.

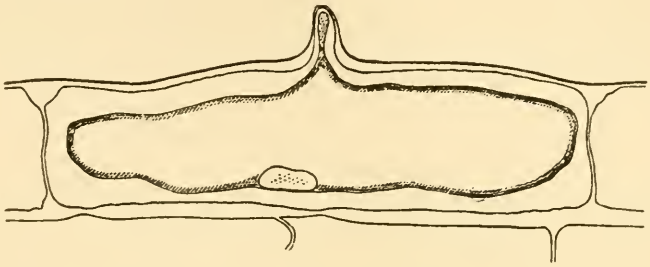

FIG. 159.—Epidermal cell of staminal filament of *Opuntia vulgaris*, showing a contact papilla. (*After Haberlandt.*)

Filaments of the Cynareæ (*Centaurea jacea*, for example) and some other groups of plants also respond to contact stimuli. They contract when weak pressure is applied, the shortening being accompanied, as in the Mimosa pul-

FIG. 160.—Two branches of *Desmodium gyrans*. *A*, in day position; *B*, in night position. (*After Darwin.*)

vinus, by an extrusion of water into the intercellular spaces. The epidermal cells of the filaments have specially sensitive papillæ with very thin cell walls, into which the protoplasm extends (Fig. 159). These papillæ are the sensitive organs that receive the stimuli.

The fully mature leaves of many plants take a different position during the day from that assumed at night. Their leaflets approach each other at night

and the petiole frequently falls. This phenomenon is called *nyctitropism* or night movement of the leaves.[b] The falling of mechanically stimulated leaflets of Mimosa is caused by decreased turgidity in the lower half of the pulvinus, but the night movement of Mimosa leaves is the result of *increased* turgidity in the *upper* half of the pulvinus. Artificial shading also causes nyctitropic movements in leaves. In Fig. 160 two branches of *Desmodium gyrans* are shown, the leaves of one in the day position and those of the other in the night position. The approach of night causes the leaves to fall and lie against one another.

Summary

1. **General Survey of Plant Movements.**—Bendings of ordinary plants (movements of one part of the plant body with reference to the rest) may be classified in two groups, *growth movements* and *variation* or *turgor movements*. Each group has two classes, *autonomic* and *paratonic* movements. Autonomic movements, whether of growth or of variation, are specifically controlled by *internal* conditions; that is, they are automatic or spontaneous, not being occasioned by changes or one-sided influences in the surroundings. Paratonic movements, of both primary groups, are always occasioned by some special change or asymmetry in the surroundings. For autonomic movements the development of the plant first prepares the mechanism for movement and then supplies the st mulus that sets the mechanism in operation. For paratonic movements the deve opmental processes prepare the mechanism, but they *do not* furnish the stimulus that sets the mechanism in operation; the stimulus must come from the environment.

Growth movements are due to unequal enlargement on the opposite sides of the bending part. Here belong the bendings due to the tropisms. They are always confined to parts that are still in the second phase of growth, the phase of enlargement. Variation movements occur in mature or maturing parts (that have ceased to enlarge). They are due to rapid changes in the turgidity of certain portions or regions of the bending organ, which set up new kinds of tissue strains and thus produce bending.

Some of the main paratonic growth movements have been considered (Chapter III, Sections 5 and 6), those due to phototropism and geotropism. The autonomic growth movements include *nutation*, *circumnutation*, the circular movements of twiners, and the *nastic* bendings. *Epinasty* is more rapid enlargement on the upper side of a leaf, resulting in a *downward* bending (as in dandelion leaves). *Hyponasty* is more rapid enlargement on the lower side of the organ, resulting in an *upward* bending.

The following scheme will help to make the classification of plant movements more easily understood.

I. Growth movements (confined to enlarging parts or organs).
   1. Autonomic growth movements (due to internal stimuli).
      (*a*) Nutation (as of bean epicotyls).
      (*b*) Circumnutation (as of most vertically elongating shoots).
      (*c*) Twining movements (as of the terminal internodes of hop, etc.).
      (*d*) Nastic bendings (epinasty and hyponasty).

[b] These movements are not properly to be considered as tropisms, for the latter are all *growth bendings*. Perhaps the best term for the day-night movements in question (which are paratonic movements of variation) is *photeolic* movements, due to alterations in light intensity.—*Ed.*

2. Paratonic growth movements (the tropisms, due to external stimuli).
   (*a*) Phototropic bendings.
   (*b*) Geotropic bendings.
   (*c*) Bendings due to other tropisms (hydrotropism, chemotropism, traumatropism, thigmotropism, etc.).

II. Movements of variation, or turgidity movements (in mature or maturing organs).
   1. Autonomic movements of variation (due to internal stimuli).
      Movements of the lateral leaflets of the telegraph plant, *Desmodium gyrans*.
   2. Paratonic movements of variation (due to external stimuli).
      (*a*) Stomatal movements, opening and closing of the stomatal pores (due to changes in light intensity).
      (*b*) Photeolic movements of leaves of legumes, Oxalis, and some Euphorbias (often called "sleep" movements; due to changes in light intensity).
      (*c*) Contact, etc., movements of sensitive plants (as *Mimosa pudica*).

2. **Autonomic Movements of Variation.**—The most striking example of autonomic movements of variation is found in the movements of the lateral leaflets of the telegraph plant, which move in such a way that the tip of each leaflet transcribes an ellipse. The movement is very rapid and quite noticeable with high summer temperatures. The terminal leaflet of red clover moves in somewhat the same way, but much more slowly.

3. **Paratonic Movements of Variation.**—The contact movements of the leaves of the sensitive plant are paratonic movements of variation, as also are stomatal movements and the night-day movements of leaves of the legumes, etc. In the contact movements and night-day movements, bending is not dependent on enlargement but is due to a rapid change in the turgidity of cells of the leaf *pulvini*, which are special bending organs. Upon being stimulated, the protoplasm of the active cells quickly increases its permeability toward the osmotic substances dissolved in the vacuoles, some of the cell sap moves into the intercellular spaces, and the tissue becomes flaccid. After a time the sap is absorbed again and turgidity is regained. Some other plants exhibit movements similar to those of the sensitive plant, but to a less marked degree. The staminal filaments of some flowers show pronounced contact responses of a similar nature.

The night-day (*photeolic*) movements of the leaves of many plant forms (leguminous plants, Oxalis, some Euphorbias) are brought about by pulvinus reactions much like those described above, but in these cases the stimulus is not mechanical contact, but a decrease or increase in light intensity.

CHAPTER VI

DEVELOPMENT AND REPRODUCTION

§1. **Influence of External and Internal Conditions on Development.**—The form and the arrangement of the parts of plants are dependent upon external conditions to a very marked degree. According to the conditions under which they develop, plants vary in their external form as well as in their internal structure, and many peculiarities in configuration that appear to be brought about solely by internal conditions are mainly the result of the external conditions that prevailed during the period of development. It has already been seen (Part II, Chapter III) that each external condition—such as heat, light, atmospheric pressure, humidity, gravitation, and the supply of nutrient material—exerts an influence upon plant growth, and consequently upon both external form and internal structure. This influence is of course more pronounced when a number of different environmental conditions affect the plant simultaneously, as is the case in nature.[a] For example, the climato-

FIG. 161.—*Achyrophorus quitensis*, an alpine plant. (⅔ natural size.)

logical conditions of high mountains are very different from those of lowlands, and alpine plants differ in a corresponding way, in form as well as in structure, from those growing at lower levels.[1] The predominating plants of high moun-

[1] **Wagner, A.,** Zur Kenntnis des Blattbaues der Alpenpflanzen und dessen biologischen Bedeutung. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien **101**^{I}: 487–548. 1892.

[a] The relations of external and internal conditions to growth and development are far too complex to be treated satisfactorily in the space devoted by the author to this aspect of physiology. In this chapter, as well as in the preceding ones devoted to growth, the author has frequently secured brevity of treatment by slighting fundamental principles and letting the presentation be largely a series of superficial, or at least incompletely analyzed, examples or illustrations from observations. Although it seems to the editor that this part of physiology really merits just as thorough analysis and just as careful thought as does respiration, for instance, still no attempt has been made to improve the analysis or to better the presentation in this chapter. A somewhat more analytical point of view is taken in the editor's very brief treatment presented in the summary. A more thorough attempt to bring out the fundamental principles of these ecologico-physiological considerations is to be found in Part II of Livingston and Shreve's book on climate and plant distribution, especially pages 97–148. (See note *a*, p. 256.)

tains have more or less reduced stems, relatively large, rigid leaves, and large, brightly colored flowers. Fig. 161 represents a specimen of *Achyrophorus quitensis*, a plant with well-developed alpine characteristics, found at altitudes of from 3000 to 4000 m. in the Andes, from Colombia to Peru.

FIG. 162.—Two plants of *Betonica officinalis*, one grown in the lowland (*P*) and the other in the mountains (*M*). (*After Bonnier.*)

The experiments of Bonnier[1] have shown that many of the peculiarities of alpine plants are due to the environmental conditions under which they grow. This author raised plants from the same lowland-grown seed in three different

[1] **Bonnier, Gaston,** Cultures expérimentales dans les Alpes et les Pyrénées. Rev. gén. bot. 2: 513–546. 1890. **Weinzierl, Theodor, Ritter von,** Der alpine Versuchsgarten auf der Vorder-Sandlingalpe bei Aussoe. und die daselbst im Jahre 1890 begonnenen Samenkultur- und Futterbauversuche. Landw. Versuchsst. 43: 27–126. 1894.

localities, near Paris, in the Alps and in the Pyrenees. Those grown near Paris had the usual appearance of their lowland parents, while those grown in the mountains had many of the characteristics of alpine forms. For example, of the two specimens of *Betonica officinalis* shown in Fig. 162, one (*M*) grew in the

FIG. 163.—Two plants of *Helianthus tuberosus* (Jerusalem artichoke), one grown in the lowland (*P*), the other in the mountains (*M*); at the right the latter is enlarged (*M'*). (*After Bonnier.*)

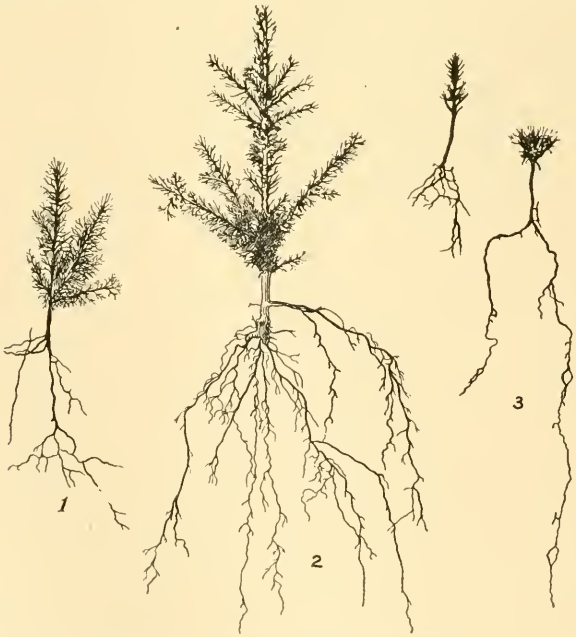

FIG. 164.—Picea (spruce) seedlings, three years old, grown under like conditions but from different stocks. 1, seed from the Achental (in the Austrian Tyrol) at an altitude of 1600 m.; 2, seed from the same region but at an altitude of 800 m.; 3, seed from Finland.

mountains and the other (*P*) in the lowland. In the mountain form the whole plant was smaller and the leaves were more crowded and nearer the base of the

stem. The difference between plants of Jerusalem artichoke (*Helianthus tuberosus*) grown under these two sets of conditions was very striking (Fig. 163). In this case the lowland form was tall, with spirally arranged leaves, and the whole plant was very similar to the common sunflower (*Helianthus annuus*), but the alpine plant, grown at an altitude of 2300 m., was quite different in appearance, being very much smaller, with almost no stem and with the leaves in a rosette close to the ground. This species is thus so strongly influenced by the climatological conditions of high altitudes that it assumes the typical form of an alpine plant even in the first generation under these conditions.

These examples show how readily the forms of many plants become altered by changed external conditions. Even in the first generation this influence of the surroundings may be very marked, and when the new set of conditions is effective throughout a number of generations the resulting changes may be inherited. Such inherited characteristics may then be retained throughout a number of generations, notwithstanding further environmental changes.[1] Fig. 164 represents spruce seedlings three years old, all grown under identical conditions, but from seed that came from different regions. Seed from trees growing under favorable conditions, at relatively low altitude (800 m.), produced very large plants (Fig. 164, 2), but seed from trees growing in the same geographic region but at higher altitude (1600 m.) produced much smaller plants (Fig. 164, 1). The plants obtained from seed that grew in Finland (Fig. 164, 3) were much smaller than any of the others.

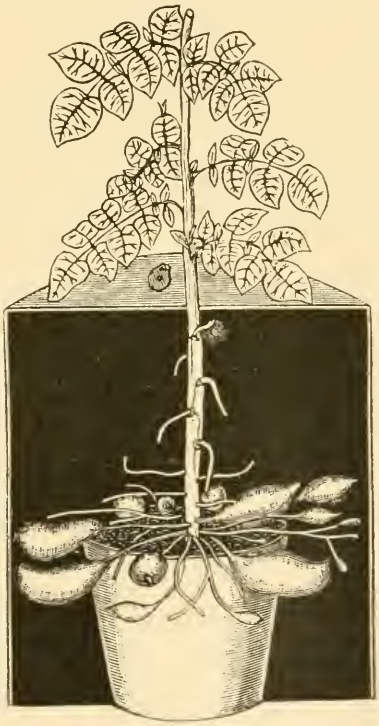

FIG. 165.—Formation of potato tubers above the soil, on darkened portion of the stem. (*After Vöchting.*)

Practical as well as theoretical importance is attached to the principle of heredity just illustrated, for to obtain a good agricultural crop not only must the soil be well cultivated and fertilized but seed of a good stock or strain must be used also.

Scientists have not been satisfied with studying the influence of external conditions in the control of form and structure of plants, but they have also been interested in discovering the genetic relationships that exist between plant organs. Until very recently problems of this sort have been attacked exclusively by the method of simple observation. From such morphological observations, the plant body (in the case of vascular plants) is considered as made up

[1] **Demoore, J.**, La mémoire organique. Bull. Soc. Roy. Sci. Méd. et Nat. Bruxelles **65**: 28–40. 1907.

of three primary parts or organs—roots, stems and leaves; all other organs not at first recognizable as roots, stems or leaves are regarded as modifications of one of these three types. Thus floral parts are considered as modified leaves. Potato tubers are a special kind of short, thick, underground stems, since they are formed on subterranean stem-branches and not on the roots. The so-called potato-eyes are dormant buds with embryonic leaves, which furnish additional evidence that the tuber is really a kind of stem. This conception arose as the result of simple observation and comparison, but experimental support therefor was furnished by Vöchting,[1] who found that potato tubers may be made to develop above ground, on branches that arise when the lower portion of the plant is deprived of light (Fig. 165).

Absence of light may therefore be considered as a condition favoring the formation of tubers, but it is not an essential condition for this, for aerial tubers may be obtained in light also. A leafy shoot is cut from the potato plant and all buds are carefully removed from the basal portion, after which the shoot is so planted in soil that there are no underground buds. Roots develop and a new plant is formed but no underground branches can develop, and consequently no underground tubers, on account of the absence of buds from the subterranean part of the stem. As the plant grows the starch that is formed in the leaves

FIG. 166.—Formation of aerial tubers from ordinary buds of the potato plant. (*After Vöchting.*)

accumulates in the ordinary buds, above the soil surface, and these develop into aerial tubers (Fig. 166). These are very similar to underground tubers, except that they are bright cherry red in color and have large eyes that bear green leaves. Under such conditions the tubers are always formed at the base of the stem, but they may be produced near the tip by placing this portion in a

[1] **Vöchting, Hermann,** Ueber die Bildung der Knollen. Bibliotheca botanica 1^4: 11-53. 1887.

dark chamber (Fig. 167). In the latter experiment the direction of the movement of organic materials through the stem occurs mainly in the direction opposite to that in which it usually occurs; these substances here move from the leaves below to the tubers above.

Vöchting[1] showed that tuber-formation in the potato is dependent also upon many other external conditions, besides those here mentioned. Aerial tubers may be similarly produced on other plants that usually bear subterranean tubers.

Because of their position in the soil, rhizomes or root-stocks, which are of frequent occurrence in plants, are often thought to be roots, but they are really subterranean stems, for they possess dormant buds that may develop later into aerial branches. Vöchting[2] has shown experimentally that this is true for *Stachys tuberifera* and *Stachys palustris*, both of which have underground rhizomes. Aerial rhizomes may be obtained with these plants by the same treatment as was employed to bring about the development of aerial tubers in the potato. If all the buds are removed from the basal portion of a cut leafy branch and this portion of the stem is then placed in soil, roots develop but no under-ground rhizomes are formed, there being no buds on the underground portion of the stem, from which rhizomes might arise. Under these conditions rhizomes do develop, however, from axillary buds on the upper portion of the stem, thus replacing the usual lateral branches (Fig. 168). Aerial rhizomes may be obtained in another way, in the plants employed by Vöchting (especially in *Stachys palustris*). If normally developed plants, with subterranean rhizomes, are brought indoors in late

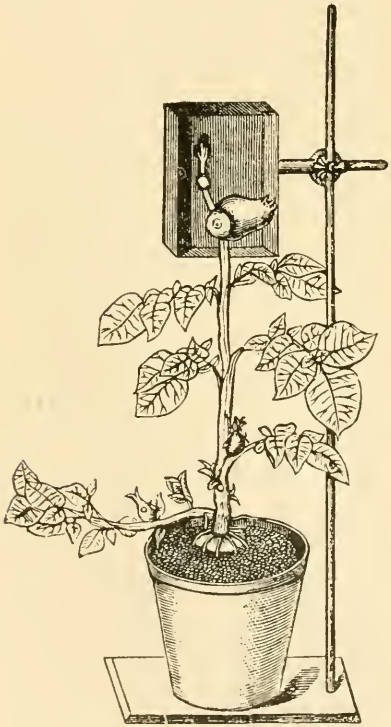

FIG. 167.—Development of terminal buds into aerial tubers as a result of darkening, by surrounding the upper part of the stem with an opaque box. (*After Vöchting.*)

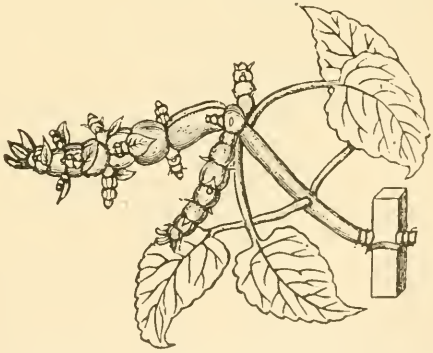

FIG. 168.—Transformation of a leafy branch of *Stachys tuberifera* into aerial rhizomes. (*After Vöchting.*)

[1] **Vöchting, Hermann,** Ueber die Keimung der Kartoffelknollen. Experimentelle Untersuchungen. Bot. Zeitg. **60**[I]: 87–114. 1902.

[2] **Vöchting, Hermann,** Ueber eine abnorme Rhizom-Bildung. Bot. Zeitg. **47**: 501–507. 2889.

autumn, when they are full-grown and are about to die, growth is resumed after a time and aerial rhizomes are produced. These experiments prove definitely that tubers and rhizomes are really modified stems, in the sense of the plant morphologists.

In the examples described above, of the experimental production of aerial tubers and rhizomes, the nutrient materials, being unable to accumulate in the usual subterranean storage organs, proceed to accumulate in the aerial stems and so transform these into storage organs. It is possible, however, to bring about the accumulation of food material in an entirely different kind of organ from that in which it usually occurs. For example, in *Boussingaultia baselloides*, which forms tubers under usual conditions, the accumulation of starch, etc., may be made to occur in the root. To accomplish this, the petiole of a cut leaf is buried in soil. Roots develop at the cut end of the petiole, and there results a simple kind of plant consisting of a leaf and roots, without any stem. The organic materials produced in the leaf accumulate in one of the roots in this case, which becomes greatly thickened and forms a tuber-like storage root (Fig. 169).

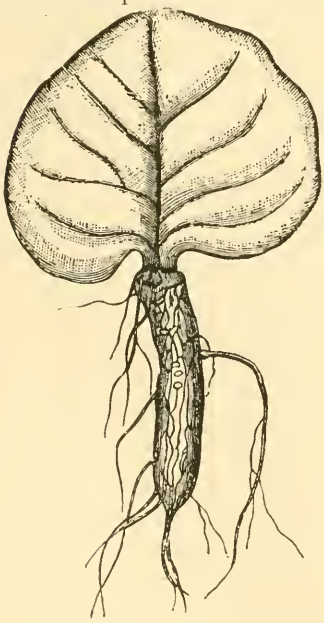

FIG. 169.—Swollen, tuber-like root, developed at the cut end of the petiole of a leaf of *Boussingaultia baselloides*.

FIG. 170.—Two segments of a willow twig, one suspended in the normal (*A*) and the other in the inverted position (*B*). *S*, stem-pole; *W*, root-pole. (*After Vöchting.*)

§2. Influence of Internal Conditions on Development.—In the physiological study of plant development and of the conditions controlling this process, *internal* as well as external conditions must of course be considered. The existence of a *polarity* in stems, for example, was demonstrated by Vöchting[1] in the following manner. Cut pieces of a willow shoot are suspended vertically in a moist chamber, some of them being inverted, so that the end that was originally toward the root (the root-pole) is now uppermost. The upright pieces form roots at the lower end and leafy shoots at the upper, while the inverted pieces develop only roots at the upper end—where these organs seem to be teleogically useless—and only branches at the lower end (Fig. 170). It thus appears that each piece of willow stem possesses two poles, a root-pole and a shoot-pole, and the tissues near each pole always produce the kind of organ characteristic of that particular pole, without reference to external conditions, such as gravitation, light, etc.

The mutual influence of various organs and their peculiar and seemingly purposeful activities in the developmental process—in fact, all that is implied in the term *consensus partium*—have long constituted an enigma in physiological science. In animals, the regulating activities by which the correlation between different organs and tissues are brought about have been, until recently, ascribed to the nervous system, but it is now known that there are special substances that control the activities of the different organs and even bring about the development of new organs. Each of these substances is formed in some special part of the organism and is then transferred to other parts, which may be at a great distance, and it may there induce various kinds of chemical reactions. Starling[2] has introduced the term *hormone* for this kind of substance, which acts, as it were, like a chemical messenger. The effect of the development of one organ upon that of another was emphasized, for the animal organism, by Brown-Séquard, who showed that there is a chemical substance in the testes of the male that affects the whole condition and even the mentality of the organism. His conclusions concerning the influence of these substances are embodied in the following quotations. "Je crois encore qu'il est parfaitement possible de réparer des ans les outrages réparables."[3] "Les testicules donnent à l'homme ses plus nobles et ses plus utiles attributs." (Brown-Séquard, 1889, page 652.)

Substances are produced in the testes by internal secretion, and these, being distributed through the body, occasion many of the pronounced differences between male and female animals. There are many illustrations of hormone action in animal physiology, of which a single instance may be referred to in detail here, the relation between pregnancy and the development of the lactiferous glands. Ribbert[4] grafted a mammary gland from one animal on to

[1] **Vöchting, Hermann,** Ueber Organbildung im Pflanzenreich. 1 and 2 Th. Bonn. 1878 and 1884.

[2] **Starling, E. H.,** The Croonian Lectures on the chemical correlation of the functions of the body. Lancet 169: 339–341, 423–425, 501–503, 579–583. 1905. Bayliss and Starling, 1906. [See note 2, p. 170.]

[3] **Brown-Séquard, C. E.,** Expérience démonstrant la puissance dynamogenique chez l'homme d'un liquide extrait de testicules d'animaux. Arch. physiol. 1: 651–658. 1889.

[4] **Ribbert, Hugo,** Ueber Transplantation von Ovarium, Hoden und Mamma. Arch. Entwickelungsmech. der Organismen 7: 688–708. 1898.

another female, near the ear, and the grafted gland enlarged during the pregnancy of the animal on which it was grafted, and even yielded milk at the end of pregnancy. Starling's experiments and those of Lane-Claypon[1] have shown that a special hormone, developed in the embryo and distributed throughout the body of the mother, is involved in the case just described. These workers succeeded in inducing the development of lactiferous glands in virgin females of the rabbit by injecting an extract of the fœtus from a pregnant female. The artificially produced gland was about as large as in the case of a normal pregnant female at about the ninth or tenth day of pregnancy.

The following quotation from Biedl[2] gives an idea of some modern conceptions concerning hormone action. "Today we find that the theory of internal secretion plays an important part in nearly all the problems of physiology and pathology and that it is very important in connection with general biological problems. Nothing is more characteristic of the recent change in our attitude toward the rôle of specific internal secretions than is Schiefferdecker's hypothesis concerning the rôle of specific internal secretions in the control of the nervous system. This hypothesis supposes that the influence upon other cells, exerted by the metabolic products emanating from nerve cells during their ordinary nutritive processes, is tropistic in nature, while the influence exerted by substances arising in nerve cells during their special activity is to be considered as a stimulating one. These conceptions of the nature of nervous control are now indeed, generally accepted, but they show very clearly how our attitude toward nerve activity has changed in recent times. All correlations in activity between organs used to be regarded as nervous phenomena, now nervous control is regarded as of a chemical nature."

Hormones probably occur also in plants, as well as in animals.[3] Even as early a writer as Duhamel gave vague expression to the idea that various phenomena of plant growth and development are not to be explained by reference to external conditions alone, and Sachs elaborated this idea and expressed the opinion that an explanation of many such phenomena must be sought inside the plant itself. In a paper on the relation of material to the form and structure of plant organs[4] this author expressed himself very definitely, stating that "with a diversity in the form of organs goes a corresponding diversity in the materials of which they are composed." Before Brown-Séquard and other authors entered this field in animal physiology Sachs had written of organ-forming materials ("*Organbildende Stoffe*"), and in his work concerning the influence of ultra-violet rays upon the formation of flowers he wrote: "These flower-forming substances act, like ferments, upon large masses of plastic material, although they themselves are present in exceedingly small amounts."[5]

[1] Lane-Claypon, (Miss) J. E., and Starling, E. H., An experimental enquiry into the factors which determine the growth and activity of the mammary glands. Proc. Roy. Soc. London B 77: 505–522. 1906.

[2] Biedl, Arthur, Innere Sekretion. Ihre physiologische Grundlagen für die Bedeutung für die Pathologie. Berlin, 1910. P. 23.

[3] Massart, Jean, Essai de classification des réflexes non nerveux. Ann. Inst. Pasteur 15: 635–672. 1901 [Idem, same title. Receuil Inst. Bot. Bruxelles 5: 299–345. 1901.]

[4] Sachs, J., von, Stoff und Form der Pflanzenorgane. Arbeit Bot. Inst. Würzburg 2: 452–488, 689–718. 1882.

[5] Sachs, Julius, Ueber die Wirkung der ultravioletten Strahlen auf die Blüthenbildung. Arbeit. Bot. Inst. Würzburg 3: 372–388. 1887. Idem, Gesammelte Abhandlungen über Pflanzenphysiologie 1: 307–309. Leipzig, 1892.*

If the word *hormones* is substituted for *ferments* in this sentence the statement becomes quite modern. Many phenomena of growth and of the developmental configuration of plants will surely be found to be dependent upon various hormones, and one of the main problems of future investigators will doubtless deal with these internal secretions of plants.

In recent years proof of the unity of all living organisms and their common genetic origin has repeatedly been adduced from physiological studies. Related organisms generally contain characteristic substances that are chemically related. Studies on animals have given important results in this connection. For instance, repeated injection of foreign blood into living rabbits leads to the formation of a special precipitin or antiserum in the rabbits' blood, and this precipitin produces coagulation in blood of the kind injected.[1] When rabbits' serum, taken from an animal thus treated, is added to the blood of other animals, the latter blood is coagulated only when these animals are of the same species as the animal from which the foreign blood originally came, or when they are closely related to that animal. Blood of species not thus closely related to the animal furnishing the injected blood, is not affected. The antiserum obtained by injecting human blood into an animal precipitates only the blood of man and of the closely related anthropoid apes (the gibbon, orang-outang, chimpanzee and gorilla) while blood of the apes of the new world is not thus coagulated. The antiserum produced by the blood of a member of the genus Canis (dog) coagulates blood of other species of this genus but not that of the less closely related beasts of prey. Similar results have been obtained also in plants. Rabbit serum from an animal that has been injected with yeast extract, precipitates extract of yeast and that of truffles, but not that of ordinary mushrooms. It therefore follows that yeasts and truffles are members of the same group of fungi (Ascomycetes). Experiments of this kind with seed-plants show that injection, into an animal, of extracts of different parts or regions of the same plant, causes the formation of the same antiserum.

§3. **Reproduction.**—The physiology of plant reproduction has been very little studied, but it is clear that this process is dependent upon both external and internal conditions. The alga Vaucheria, for example, consists of a long, unicellular filament that reproduces both sexually and asexually. In asexual, or vegetative, reproduction the terminal portion is separated from the remainder of the filament by a dividing wall. The cell thus cut off is the zoosporangium, from which the zoospore escapes as a many-ciliated, motile cell. After a period of free movement, this cell enlarges and grows into a filament like its parent, thus forming a new individual. The process of zoospore formation is markedly influenced by external conditions, as has been shown by Klebs.[2] Vaucheria may be grown indefinitely without forming zoospores, or zoospores may be produced at any time, according to the desire of the experimenter. Zoospores never develop when the cultures are kept in moist air, but the filaments

[1] **Seber, M.,** Moderne Blutforschung und Abstammungslehre. Frankfurt a. M., 1909.* **Ballner, Franz,** Ueber die Differenzierung von Pflanzlichem Eiweiss mittels der Komplementbindungsreaktion. Sitzungsber. (math.-naturw. Kl.) K. Akad. Wiss. Wien 119^{III}: 17–58. 1910.

[2] **Klebs,** 1896. [See note 1, p. 300.]

need only to be transferred to water to bring about the formation of these special cells. They continue to be produced for some time under these conditions, but finally the process ceases even in water. If the water culture is then removed from light to darkness, zoospore formation begins again, and by transferring such a culture back and forth, between darkness and light, it is possible to call forth this reproductive response or to check it, at will. If the plant is grown in water without the requisite mineral salts, the power to form zoospores is lost and does not reappear, even if the culture is transferred to darkness, unless the essential nutrient salts are re-supplied.

In sexual reproduction each Vaucheria filament usually develops two lateral outgrowths, one of which forms the antheridium while the other becomes the oogonium. The egg cell of the mature oogonium is fertilized by one of the numerous sperms liberated from an antheridium, and the oospore formed by this union develops into a new individual, the whole process constituting sexual reproduction.

Sexual reproduction in Vaucheria is dependent upon external conditions. Adequate light conditions and the presence of carbon dioxide in the solution or in the air about the cells, are necessary for the production of sex organs, for the ordinary processes of nutrition must continue during the formation of these organs. No sexual organs are formed in light when carbon dioxide is lacking, unless, indeed, the lack of the latter is supplied by sugar in the solution. Absence of light cannot be thus counteracted by the presence of sugar, however. When the culture medium contains sugar and the atmosphere is without carbon dioxide, antheridia and oogonia are formed in light and not in darkness. Vaucheria filaments may be so treated that they are unable to reproduce sexually, even when illuminated. If the culture is grown for a comparatively long time in a sugar solution, in weak light or in darkness, the cells become gorged with oil and lose the power to reproduce.

Finally, the quantitative relation between the number of oogonia and the number of antheridia may be modified by altering the external conditions. There is generally one oogonium for each antheridium in *Vaucheria repens*, for example, though less frequently there may be one antheridium for each two oogonia. The number of oogonia formed may be reduced, while the number of antheridia may be greatly increased, by subjecting the plants to high temperature or to much reduced atmospheric pressure. As many as five antheridia in a group, without any oogonia at all, may sometimes be formed with this treatment.

In connection with the study of sexual reproduction the question arises as to what may be the conditions determining the entrance of the sperms into archegonia or oogonia. To attack this problem experimentally, very fine capillary glass tubes filled with various solutions are laid in a drop of water containing the sperms to be studied. According to the nature of the solutions diffusing from the open ends of the tubes and according to the kind of sperms present, the latter are either attracted in large numbers and swim into the tubes, or they are not affected at all. It appears that each species of sperm is attracted

more by certain substances than by others; fern sperms are strongly attracted by malic acid and still more attracted by the common soluble salts of this acid, while moss sperms are most attracted by cane sugar. There appears to be no doubt that the maturing moss archegonia secrete a special substance that attracts sperms of the same species. Upon the sperms of other plants this substance appears to have no effect.

The reproduction of fungi is also influenced by a large number of external conditions.[1] It is generally true that reproduction does not occur in algæ and fungi under conditions favorable to vegetative growth, while conditions favoring reproducton usually retard vegetative growth.[2]

Sexual consanguinity[b] is necessary for the union of the sexual cells of seed-

Fig. 171.—Germinating pollen-grains of *Vallota purpurea*, their tubes directed toward a mass of diastase. (*After Lidforss.*)

plants as well as of spore-plants. The chemotaxis of sperms (as of ferns) is paralleled by the chemotropism of the pollen-tubes of flowering plants. Just as the sperms swim toward the source of diffusion of the attracting substance (such as malic acid), so do the pollen-tubes bend and elongate toward this source. Fig. 171 shows a culture of pollen-tubes of *Vallota purpurea* growing in a 30-per

[1] **Klebs, Georg,** Zur Physiologie der Fortpflanzung einiger Pilze. III. Allgemeine Betrachtungen. Jahrb. wiss. Bot. 35: 80–203. 1900.

[2] Also, see: **Jickeli, Karl, F.,** Die Unvollkommenheit des Stoffwechsels als Veranlassung für Vermehrung, Wachsthum, Differenzierung, Rückbildung und Tod der Lebewesen im Kampf ums Dasein. Berlin, 1902.

[b] This and the next following paragraph are added from the 7th Russian edition.—*Ed.*

cent. solution of sugar with gelatine enough to form a jelly. The dark area in the center represents a mass of diastase, toward which the growing filaments are attracted.[1]

It appears that there are substances in the flowers of some plants that prevent fertilization by pollen from the same individual, thus resulting in self-sterility. If the pollen is applied to the stigmas of another individual of the same species, however, fertilization is not thus prevented.[2] In such cases the pollen, to be effective, must be applied to an individual of different sexual origin from the one that bore it.

Parthenogenesis is also controlled by external conditions. Nathansohn,[3] for instance, succeeded in obtaining parthenogenetic reproduction in several species of the genus *Marsilia* by subjecting the spores to high temperatures.

Higher plants may propagate themselves vegetatively, by means of tubers, bulbs, etc. An organ, or even a portion of an organ, removed from the plant, may generate a new individual.[4] For instance, if a Begonia leaf is cut off and laid upon moist sand, adventitious roots are formed and a new leafy branch develops. If the leaf is taken from a plant that is in bloom, the branch that develops bears flowers instead of being leafy. Fig. 172 shows a leaf of *Achimenes haageana* that was taken from a plant just about to bloom; flowers have been developed instead of leaves.[5] Leafy branches or flowers may be obtained at will, by cutting the leaves for propagation from plants in the proper stage of development. It thus appears that the leaves of a plant about to bloom contain different chemical substances from those found in the leaves of earlier developmental stages.[6]

The ancient Greeks were already aware that if a bud is taken from one plant and grafted upon another a new branch is produced by the development of the bud, and that this branch retains the special character of the plant from which the bud originally came. The operation of grafting, known to gardeners for so long a time, furnishes the physiologist with a valuable means for studying the processes of growth and metabolism. Vöchting[7] has collected the scattered

[1] **Lidforss, Bengt,** Untersuchungen über die Reizbewegungen der Pollenschläuche. Zeitsch. Bot. **1**: 443–496. 1909.

[2] **Correns, C.,** Selbststerilität und Individualstoffe. Festschr. (84 Versamml.) Deutsch. Naturf. u. Aerzte, med.-naturwiss. Ges. P. 186–217. Münster i. Westf., 1912.

[3] **Nathansohn, Alexander,** Ueber Parthenegenesis bei Marsilia und ihre Abhangigkeit von der Temperatur. Ber. Deutsch. Bot. Ges. **18**: 99–100. 1900.

[4] **Goebel, K.,** Ueber Regeneration im Pflanzenreich. Biol. Centralbl. **22**: 385–397, 417–438, 481–505. 1902. [In this connection see also; **Loeb, Jacques,** Rules and mechanism of inhibition and correlation in the regeneration of Bryophyllum calycinum. Bot gaz. **60**: 249–276. 1915. **Idem,** Further experiments on correlation and growth in Bryophyllum calycinum. *Ibid.* **62**: 293–302. 1916. **Idem,** On the association and possible identity of root-forming and geotropic substances or hormones in Bryophyllum calycinum. Science, n. s. **44**: 210–211. 1916. **Idem,** Influence of the leaf upon root formation and geotropic curvature in the stem of Bryophyllum calycinum, and the possibility of a hormone theory of these processes. Bot. gaz. **63**: 25–50. 1917. **Idem,** A quantitative method of ascertaining the mechanism of growth and of inhibition of growth in dormant buds. Science, n. s. **45**: 436–439. 1917. **Idem,** The chemical basis of regeneration and geotropism. *Ibid.* **46**: 115–118. 1917. **Idem,** The organism as a whole. X + 153 p. New York, 1916.]

[5] **Goebel, Karl E.,** Organographie der Pflanzen, inbesondere der Archegoniaten und Samenpflanzen. Jena, 1898–1901. Part I, p. 41. [**Idem,** Organography of plants especially of Archegoniatæ and Spermaphyta. Translated by Isaac Bayley Balfour. 2v. Oxford, 1900–1905.]

[6] **Klebs, Georg,** Ueber die Nachkommen künstlich veränderter Blüthen von Sempervivum. Sitzungsber (math.-naturw. Kl.) Heidelberg. Akad. Wiss. 1909[6]: 1–32. 1909.

[7] **Vöchting, Hermann,** Ueber Transplantation am Pflanzenkörper. Tübingen, 1892.

literature of this subject and has employed the surgical term *transplantation* to designate all kinds of coalescences between plant parts.

Experiments have shown that widely different portions of plants may be brought together and made to coalesce. Even the transplantation of a leaf directly on to a root may be accomplished, as in the case of the beet. The whole upper portion of a beet plant is cut away, leaving nothing but the fleshy root, in the lower portion of which an incision is made. Into this incision is inserted the cut end of a leaf petiole and the two parts are bound together. The tissues coalesce and the leaf remains alive and grows.[1] Even portions of different varieties of fruit may be made to coalesce in this way. For example (Fig. 173), a gourd fruit of the variety *poire verte* was grafted by its stem upon one of the variety *à fruits jaunes;* then the lower part of the former was cut away and a similar portion of a fruit of a third variety, *à fruits blancs*, was trans-

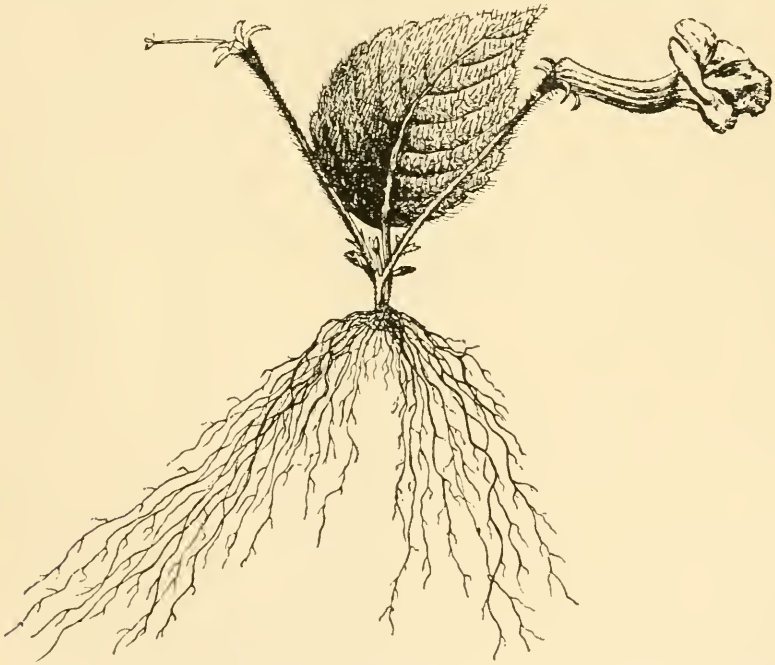

FIG. 172.—Leaf of *Achimenes haageana*, from which roots and flowers have been formed. (*After Goebel.*)

planted to the cut surface thus left. The whole system of three different kinds of fruit continued to grow after the operation.

One of Vöchting's experiments[2] illustrates how this sort of operation may

[1] **Daniel, Lucien,** Recherches morphologiques et physiologiques sur la greffe. Rev. gén. bot. **6**: 5–21, 60–75. 1894. **Idem,** Sur quelques applications pratiques de la greffe herbacée. **Ibid. 6**: 356–369. 1894. **Idem,** Un noveau procédé de greffage. *Ibid.* **9**: 213–219. 1897. **Idem,** Les conditions de réussite des greffes. *Ibid.* **12**: 355–368, 405–415, 447–455, 511–529. 1900. **Doroféjew, N.,** Ueber Transplantationsversuche an etilierten Pflanzen. (Vorläufige Mitteilung.) Ber. Deutsch. Bot. Ges. **22**: 53–61. 1904.

[2] **Vöchting, H.,** Ueber die durch Pfropfen herbeigeführte Symbiose des Helianthus tuberosus und Helianthus annuus. Sitzungsber. K. Preuss. Akad. Wiss. Berlin. **1894**: 705–721. 1894.

furnish evidence concerning the chemical processes in plants. In this case the leafy stem of a young sunflower plant (*Helianthus annuus*) was cut off a short distance above the soil and to the cut surface of the stump was grafted a leafy branch of the Jerusalem artichoke (*Helianthus tuberosus*). Union of the two parts soon occurred and a new plant was formed. Examination of the sap showed that the upper portion, down as far as the plane of the graft, contained inulin in abundance, while the part below the plane of the graft contained starch but no inulin. In this case the simple organic substances in the sap of both portions were produced in the artichoke leaves above. In the reverse experiment, where the upper part was sunflower and the lower Jerusalem artichoke, a similar result was obtained; namely, that starch but no inulin was present in the sunflower portion while the artichoke portion, which here received its simple organic substances from the sunflower leaves, contained an abundance of inulin

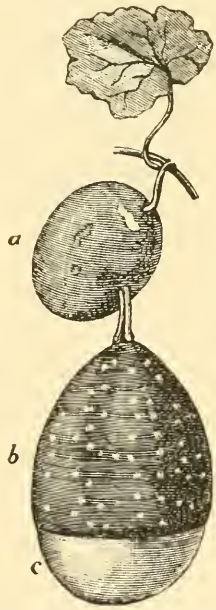

FIG. 173.—Three varieties of gourd grafted upon one another; *a, à fruits jaunes; b, poire verte; c, à fruits blancs.*

and even bore tubers, in which inulin accumulated in the same way as if the whole plant had been of the artichoke species. Inulin clearly acts only as a reserve carbohydrate. In both experiments the products of photosynthesis were present in both stem and roots as glucose, but within the limits of the sunflower portion they accumulated as starch, while within the limits of the artichoke portion they accumulated as inulin.

The operation of transplantation is successful only when closely related species are involved, as may be understood from the foregoing discussion (page 329) of hormones and of the chemical differences between the metabolic substances of forms not closely related.

Summary

1. **Influence of External Conditions on Development.**—The development of a plant is of course made up of all of its growth activities considered together. The external or internal conditions that influence growth also influence development. As the plant develops, its internal conditions (its physiological characteristics) are continually changing, the conditions of the natural surroundings are also always in a state of fluctuation, and consequently the *relations* between internal and external conditions are likewise continually varying. It is these relations that really determine the developmental processes. With a given kind of internal complex, a certain set of environmental conditions would produce a certain kind of growth. If *either* the internal or external conditions were markedly different, the kind of growth would be correspondingly different. Thus, different species in the same environment develop differently, and different individuals of the same species, but in different environments, also develop differently. Finally, present internal conditions, or characteristics, are the results of past internal and the past environmental conditions, acting together. These relations are somewhat complex, but it is clear that we may not say that any plant response, or any form of development, etc., is exclusively brought about by either the internal or the external conditions. Both sets of conditions are of course necessary for growth, and the two sets always act simultaneously.

Environmental complexes that are favorable to the development of one kind of plant are not favorable to that of another, sufficiently different, kind. Environmental complexes may therefore be said to be *adapted* to the development of those plants that can thrive under their respective influences. Thus, American desert conditions are very delicately and nicely adapted to the growth and reproduction of certain kinds of cacti and other spiny shrubs, but they are not at all suited to the development of the spiny roses found growing plentifully in the more humid regions of North America. The conditions of the humid regions, on the other hand, are well adapted to the development of these roses, but are not adapted to the development of the desert cacti. The present conditions of desert and humid regions have been brought about by a long evolutionary series of climatic and physiographic changes, leading directly to the present characteristics of these regions, a series of changes that began long before there were any plants.

In a similar way, plants that thrive with one set of environmental conditions do not thrive at all under another, sufficiently different, set. It follows that plant forms may be said to be *adapted* to the particular environmental complexes under which they thrive. The internal conditions characteristic of existing plant species have been brought about by a long series of evolutionary steps, leading directly to the present species, a series of steps that began with the inception of terrestrial life, long after the corresponding climatic and physiographic series of evolutionary changes had been started on its predetermined way.

It should be added that physiographic and climatic evolution has, in some cases, been greatly influenced by organisms, and that plant evolution has always been influenced by physiographic and climatic evolution; the two lines of evolution are interwoven, and they have operated together to bring about present environments and existing plants.

While each species or form of plants requires for its development climatic and soil conditions that lie within certain definite limits, each can thrive under any one of a number of rather different environmental complexes, so long as all of these lie within

the fixed limits for that form. When two environmental complexes are different to a considerable degree but both are suitable for the development of a given species, development with one complex may be very different from that with the other. Thus, Bonnier's experiments showed that the same plant (Jerusalem artichoke, for example) developed very differently in lowlands and in alpine regions. Also, seed of the same kind of plant, each lot grown under a different set of climatic conditions, may produce very different plants when all lots are germinated together and the seedlings are reared side by side. This point is of agricultural importance.

Potato tubers are branch stems that develop underground. In darkness, tubers may be caused to develop above the soil surface. Vöchting was able to arrange conditions so that tubers were produced above the soil and in light, even at the tip of the shoot, in which case the carbohydrates formed in the leaves must have moved *upward* through the stem to the tubers. The same author accomplished similar results with rhizome-bearing forms, as well as with tuberiferous plants. If the organs in which starch usually accumulates are removed, this substance may be made to accumulate in organs not usually serving as places of accumulation. If it usually accumulates in a subterranean tuber, it may be made to accumulate in aerially formed tubers, or in roots, etc.

2. **Influence of Internal Conditions on Development.**—Each part of the plant body influences the development of other parts, and these internal influences (called correlations) are in many cases so marked that they cannot be readily overcome, if that is possible at all, by altering the external conditions. Correlations appear to be due to small amounts of specific substances produced in the cells and influencing the growth and development of other cells that are often situated at a great distance. Such growth-controlling internal secretions are well known to occur in animals and they have been called *hormones*, or chemical messengers. It seems probable that similar substances occur in plants.

3. **Reproduction.**—Reproduction is a special kind of growth. In sexual reproduction, among the various organs appearing in a mature individual are the reproductive organs, in which are produced the reproductive cells (eggs and sperms). The formation of these organs, like that of other organs, is controlled by internal and external conditions acting together. Thus, Klebs was able to secure oogonia or antheridia, or both, by proper treatment of the right form of Vaucheria. With the right treatment, this alga could also be made to live indefinitely, reproducing by zoospores and by branching, without the formation of sexual organs at all. Sperms of algae, mosses, and ferns, are attracted to the archegonia or oogonia of the same or of a similar species by certain substances that diffuse (dissolved in water) from these organs. The sperms are said to be *positively chemotropic* toward these substances. Pollen tubes of higher plants are similarly attracted by substances formed in the stigma, etc.; in this case the tubes bend because of *chemotropism*, this being a growth bending, due to unequal elongation on the opposite sides of the organ.

In some cases egg cells develop into new individuals without fertilization, this phenomenon being *parthenogenesis*. By proper treatment of the unfertilized eggs, parthenogenesis may be artificially induced in some forms in which it does not usually occur.

It is common in plants for parts of the body to separate from the rest and develop into new individuals, without any fusion of cells. This is asexual reproduction. In Vaucheria, for a simple example, the terminal portion of the protoplasm of a filament becomes a zoospore, which, after moving about for a time, simply grows into a new

plant. By proper treatment Klebs was able to produce zoospores in this alga at will, or to grow the plant without their being formed.—Higher plants reproduce asexually by means of outgrowths of various kinds, such as tubers, bulbs, etc. A branch, a leaf, or a piece of root, may thus act as a reproductive organ. Many cultivated plants are propagated by cuttings; advantage is taken of asexual reproduction in order to maintain valuable varieties, which would be lost in many cases if propagated by seeds.

Related to propagation by cuttings is propagation by grafting or transplantation. A bud or branch (*scion*) cut from one plant and inserted on another (*stock*) develops on the new stem or root, retaining all the characters of the plant from which the bud or branch was taken. Widely different parts of plants may be joined by transplantation. In general, transplantation is possible only when closely similar species or forms are involved.

INDEX

NOTE.—This list includes most of the more important topics considered in the book, embracing physiological terms, names of substances (even when used only as reagents), and genetic names of plants. Some analysis is attempted for a few topics; spatial limitations preclude more complete analyses. Authors' names are also included, with brief characterizations of the subject considered. Plant names are in *Italics*, authors' names in **black-face** type. Page numbers are in black-face type, (1) when the topics are chapter or section headings, and (2) when they refer to *full citations;* where authors are mentioned without complete citations, page numbers are in ordinary type. A dash indicates all intervening pages between the number preceding and that following.—*Ed.*

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